THE EFFECTS OF LARVAL COMPETITION ON A QUANTITATIVE CHARACTER IN *DROSOPHILA MELANOGASTER*

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SUMMARY

It is commonly assumed that quantitative characters, such as sternopleural chaeta number in Drosophila melanogaster, are under the control of stabilizing selection. The evidence for this type of selection is reviewed and it is concluded that competition at the larval stage in D. melanogaster may explain the observed relationship between chaeta number and fitness. Two extreme models of selection are described.

The environmental effects of larval competition on developmental time, body weight and survival were investigated. Intense larval competition is found to have a differential effect on the depression of chaeta number.

The concept of density-dependent selection was tested over several generations using four visible mutants. It is concluded that density did not influence the decline in frequency of these mutants except in one case.

Density-dependent selection was investigated in its effect on the quantitative character using populations constructed from a high and a low selection line. One of these populations which was segregating for high and low chaeta genes on the third chromosome did result in a change which could be interpreted as evidence for an association with larval viability. By comparing homozygous lines constructed from this population, the association was not found to be due to a pleiotropic effect of the chaeta genes but to the presence of sub-vital genes in one of the selected lines.

Since the behaviour of inbred selection lines may give misleading results in the context of 'natural' selection, two recently isolated

wild populations were studied. Lines from these populations and from the standard Kaduna population were selected for chaeta number in both directions and then relaxed. It is concluded from the magnitude of the returns to the unselected mean that chaeta number is not closely associated with fitness.

In the final chapter the results obtained in this study are discussed in relation to other findings. It is concluded that under the conditions used, chaeta number is not associated with larval viability.

CHAPTER ONE

INTRODUCTION

One of the interesting questions of population genetics today is the problem of explaining the existence of large amounts of genetic variation found in both animal and plant populations (Harris, 1966; Lewontin & Hubby, 1966; Murray, 1972). The genetic variation observed in single loci may also be expressed in variation in many quantitative characters. For example body length in pigs (Fredeen & Jonsson, 1957), wool length in sheep (Morley, 1955) and egg weight in poultry (Lerner & Cruden, 1951) show large amounts of genetic variation which has been exploited through animal breeding for a long time. The study of such characters in relation to their behaviour in selective processes is important both from an evolutionary standpoint and from the economic basis of animal breeding.

The existence of large amounts of genetic variation poses two questions, why does it exist and how is it maintained?

The answer to the first can only be of a speculative kind. There must be sufficient differences between or within organisms to ensure survival of at least part of the population, since environments will not remain constant but will fluctuate in conditions over short and long time periods. This seems to hold true for outbreeding species but haploid and inbreeding species would appear to contradict this hypothesis. However, Allard, Jain & Workman (1968) have demonstrated that extensive genetic variation is found in highly inbred populations of certain species of grasses. This variation exists between individuals revealing populations containing a large collection of unique genotypes.

In answer to the question of how this genetic variation is maintained, many explanations have been put forward, of which only the most important will be mentioned.

Mutation, which is the fundamental source of all variation, continually produces variants, which may accumulate within the genome. Most mutations are deleterious in effect and will be eradicated from the population, but there may be a large group of mutations which are selectively neutral. A balance is attained between variation produced by mutation and variation lost through random drift. This explanation has become known as the Neutral Mutation-Random Drift Hypothesis and is mainly attributed to Kimura, (see Harris, 1971, for a review). Clayton & Robertson (1955) suggested that the variation observed in sternopleural chaeta number in Drosophila melanogaster could be explained in this way.

Alternatively selection may actively maintain genetic variation within populations. Several models of selection have been described such as frequency-dependent selection (see Murray 1972, for a review), disruptive selection (Mather, 1955, and Thoday & Gibson, 1970), and stabilizing selection (Mather, 1953). Of these, stabilizing selection has been used more commonly as a model to account for the variation in quantitative characters. Waddington (1953) called this model of selection normalizing while Falconer (1964) referred to it as centripetal. This was first mentioned by Schmalhausen (1949), "Individuals near the mean of the population reproduce themselves but the extremes do not". Lerner (1958) defined it as "Individuals close to the average for a metric trait are favoured by natural selection over those far removed from the mean value of the population". Haldane (1954)

pointed out that it is likely that almost all natural selection for a quantitative character is of this type. The classic example cited is that of Bumpus (1899), who measured the wing length of sparrows which had died after a severe storm and compared them with the surviving population. The birds which had died were those with the most extreme wing measurements.

Much work has been published on this type of selection, such as clutch size in birds (Lack, 1954; Perrins, 1964), human birth-weight (Karn & Penrose, 1951; Jayant, 1966), quantitative characters in barley, wheat and lima-beans (Allard & Jain, 1962), sternopleural chaeta number in Drosophila (Kearsey & Barnes, 1970), development time in Drosophila (Prout, 1962). These observations have all shown that intermediates for these metrical characters are fitter than the extremes. However, it is very difficult to explain the underlying relationship between cause and effect for the above observations. There are two extreme explanations which have been put forward:-

i) The relationship is between the phenotypic deviation of the character per se and fitness. The fitness of an individual will decline as its deviation from the intermediate value increases. There is additive gene action on the metric scale, but an interaction between gene loci on the fitness scale conferring maximum fitness on the intermediate value. The relationship between the measurement and fitness is considered as an aspect of the phenotype. This has been referred to as the optimum model.

ii) The relationship is between the underlying heterozygosity or homozygosity of genes controlling the quantitative character and fitness. It is assumed that the genes are acting in a purely additive

manner for the metric character with no interaction. Also it must be assumed that there is over-dominance for fitness. In contrast with the optimum model this is a genotypic relationship. This is commonly referred to as the heterozygous or homeostatic model.

Robertson (1956) has worked out the theoretical consequences of the optimum model for a single locus and concludes that gene frequency will tend to 0 or 1, whereas Gale & Kearsey (1968) state that stable equilibria can be attained under the optimum model with unequal effects at different unlinked loci. However, if it is the underlying properties of the genes themselves which determine the fitness, then as Robertson pointed out, the attainment of stable equilibria is one of the basic premises of the model. This applies only to very large population sizes. When small population sizes are considered, heterozygote superiority at best can only retard the rate of fixation rather than maintain a stable equilibrium, (Robertson, 1962). Robertson (1955) is assuming over-dominance for fitness in his arguments as defined by Lerner (1954):- 'The inheritance of metric traits may be considered, at least operationally, to be based on additively acting polygenic systems while the totality of traits determining reproductive capacity and expressed as a single value (fitness) exhibits overdominance'. Bulmer (1971) also agrees that stable equilibria can only be maintained if there is overdominance at all loci when assuming a model of stabilising selection.

Jinks (1955), Mather (1955a), and Jinks & Morley Jones (1958) would argue that overdominance for metrical characters arises from epistasis and/or dispersed dominant genes. If it is assumed that genes for metrical traits have the same properties as major genes, then it

should be possible to fix this superiority in a homozygote. Breese & Mather (1960) were able to obtain isogenic lines which had the same fitness as the complete heterozygote, and therefore it would seem that heterozygote advantage could not maintain genetic variability. However, several investigations have reported the maintenance of genetic variation through heterozygote advantage, for instance sickle cell anaemia in man (Allison, 1954), transferrin polymorphism in pigeons (Frelinger, 1972) and tetrazolium oxide polymorphism in Drosophila (Richmond & Powell, 1970).

Robertson (1970) described an experiment which distinguished between the two models using the character sternopleural chaeta number in Drosophila melanogaster. Populations were constructed from a wide cross between two highly selected lines, High (H) and Low (L) with means of 49 and 9 respectively. Two populations were set up in such a way that the third chromosome was segregating for high and low sternopleural chaeta number in alternative backgrounds of high and low as indicated:

	F_2: Female Genotypes			
H / 1 / H	H / 2 / H	L / 3 / H	H / 4 / H	Blue population $\bar{X} = 28.0 \pm 0.9$
L / 1 / L	L / 2 / L	L / 3 / H	L / 4 / L	Red population $\bar{X} = 11.6 \pm 0.09$

Robertson argued that if the optimum model was true, alleles for low chaeta number would be at an advantage and increase in frequency in a high background and vice versa. This would imply some degree of frequency dependent selection. On this model it would be expected that the mean score in the Blue population would decrease and that of the Red population to increase. After several years of maintenance with large population sizes (c.5,000) no tendency of the mean scores to change was found. The other alternative populations were also set up which were segregating for chromosomes I, II & IV with the third homozygous. Again there was no tendency for the means to return towards the base mean. Recently Robertson has selected upwards in the Red population with an immediate response indicating that sufficient genetic variation still remains for such a change to take place. This type of evidence would not support the optimum model of stabilizing selection.

Again using the Kaduna population, Latter & Robertson (1962) found, that on selecting for sternopleural chaeta number in both directions fitness as measured by competitive index (Knight & Robertson, 1957) was reduced relative to control lines. On relaxation of these selection lines at generation 5 it was found that the mean of the high lines changed little over 25 generations. At later stages of selection no evidence of a return to the unselected level was found. The low lines, when relaxed at generation 5, returned to the unselected level by 50% from the response previously obtained. At later stages of selection, relaxation continued to produce a return to the unselected level. It was suggested, that loci controlling the response in the primary character, have also controlled the fitness changes in the

lines selected for low chaeta score.

Robertson (1967) gives similar results to those of Latter & Robertson (1962) for high selection lines. Three lines were selected upwards for six generations and in this time differed by about six chaetae. It is doubtful whether many of the genes influencing chaeta number would have become fixed in this time. The lines were then split into two groups, the first being raised in mass culture in population cages and the second group being selected downwards. The second group responded immediately to downward selection and went down further than the population mean in all three lines. After two years (approximately 35 generations) the means of the first group had changed by only one chaeta. These lines in the population cages were then subjected to downward selection and they all responded immediately. Robertson (unpublished) has carried out the same experiment but this time selecting for low chaeta number for six generations. On relaxation little return was observed after many generations.

On the other hand Mather (1961) found evidence of differential larval survival in the comparison of the competitive abilities of various chaeta phenotypes from a cross between two inbred lines. The chaeta scores nearest the F_1 had the highest competitive abilities.

Barnes (1968) found, that when a population constructed from a cross between two inbred lines, was kept at temperatures of $18^{o}C$ and $25^{o}C$, different equilibrium values of chaeta number were observed. Individuals at these equilibrium values were found to leave on average more offspring than individuals further from the equilibrium values. Barnes concluded that natural selection had a differential effect at the two different temperatures. Barnes claims that this evidence

suggests, that chaeta number and fitness are functionally related, variation in chaeta number being accompanied by variation in fitness.

Barnes & Kearsey (1970) and Kearsey & Barnes (1970) constructed a population from a cross between a high and a low selection line, originally selected from a wild Texas population. This cross generated a large range of chaeta phenotypes with a variance of around forty. It was observed that the phenotypic variance of adults, reared under competitive cage conditions, was about one quarter that of their contemporaries raised at non-competitive levels. It was shown by regression techniques, that this decrease in phenotypic variance was due to a decrease in the genetic variance. They claimed that the selective elimination of extreme genotypes at the pre-adult stage was related causally to chaeta number. Thus, fitness was shown to be greatest for phenotypes with a value close to that of the F_1 between the selected lines and declined markedly with deviations from this optimum. Kearsey & Barnes also quote chaeta number values for seven wild populations and comment on the similarity of their means and variances. This similarity, they suggest, indicates that natural selection is of considerable importance.

Further evidence (Linney, Barnes & Kearsey, 1971) indicated, that the results previously found, could not be explained on the basis of linkage of sub-vital genes to chaeta number genes. The original base population, Texas, was used in this investigation and the results confirmed the previous findings. To test the optimum model of selection a population of homozygous lines was reared under competitive and non-competitive conditions, and the genetic consequences were followed. The homozygous lines were derived from the Texas population

and four were chosen in such a way that the resulting population would contain members representing all chaeta classes found in the Texas population. On progeny testing the survivors from low and high density treatments in the same environment, it was found that the estimated genetic variance had been reduced under high density conditions, indicating the elimination of extreme chaeta scores. Thus the larger the deviation of inbred lines in chaeta scores from the original population mean, the less fit are these lines. It is pointed out that deleterious genes could have become fixed in the two extreme lines by chance, but this is thought to be a rather dubious assumption. This is substantial evidence to support the optimum model.

Killick (1970) found that on crossing two inbred lines the mean population value of chaeta number moved out of the parental range over a period of 25 generations of maintenance in a population cage.

McGill & Mather (1972) put forward further evidence of stabilizing selection. They crossed two wild-type lines and compared the competitive abilities of flies with different numbers of sternopleural chaetae against a common tester stock. It was found, that progeny with eighteen chaetae competed most successfully with the tester stock, competitive ability falling away as the number of chaetae decreased or increased from this value.

There are several inconsistencies in these results. Barnes (1968) found differences in number of progeny left by different chaeta classes, whereas Barnes & Kearsey (1970) could find little difference in number of progeny from their derived Texas population. These authors commented on the fact that the two lines used by Barnes (1968) have widely

different origins, and hence the population produced was artificial. Therefore the selective effects which were demonstrated may have little relevance to natural populations. Killick's result is in marked contrast to that obtained by Barnes (1968). Since only one cage was set up, contamination cannot be ruled out. McGill & Mather's results could easily be explained on a heterotic basis, although neither of the original parental scores were given. The supposition, which is made by inference from the relationship between phenotypic observations and overall fitness, that selective forces are maintaining variation in a quantitative character such as chaeta number, is very dubious.

Robertson (1955, 1964, 1967, 1970) has repeatedly stated that this approach is conceptually meaningless. His main point is that such a relationship is an effect of the developmental process and is itself a consequence. It is not possible to split up organisms into compartments as regards quantitative characters and proceed to talk of selection acting only on a particular character. Selection 'acts' on the whole phenotype (the entire life history) and not on abstractions such as chaeta number. Robertson (1967) states that "We have to know not only the type of gene action controlling the measurement, but also the way in which the genotypes affecting the measurement control reproductive fitness".

Kearsey & Barnes (1970) pointed out that the selective elimination of extreme phenotypes within their populations could not have taken place through selection against chaeta number itself, as this character is formed only after selection has taken place. They concluded that chaeta genes may have pleiotropic effects, which are

important during the larval stage of growth. Thus chaeta genes may reflect some character which is important for survival during the larval stage. One could envisage feeding rate of larvae being an important character for larval survival (Bakker, 1961). There will be several stages in the life history at which selection will act, the magnitude depending on the environmental circumstances. Hatchability (Kearsey & Kojima, 1967), larval survival (Bentvelzen, 1963), mating ability, male and female fertility (Knight and Robertson, 1957) are regarded to be the most important stages at which selection will take place. Many attempts have been made to estimate the selection coefficients of these components of fitness. Bundegaard & Christiansen (1972) found that the most important component of selection for the maintenance of a fourth chromosome polymorphism in D. melanogaster was male mating ability. Moree & King (1961) investigated the survival of the mutant ebony body when competing with its wild type allele in a Drosophila population. They found that larval survival was the largest component of selection.

As a first step in this investigation it was thought worthwhile to try and explain some of the more obvious differences in results cited previously.

An apparent difference is to be found in the maintenance of populations. The system described by Barnes (1968) is based on a continuous supply of food medium to the population. Small amounts of food are placed in a population cage every alternate day, replacing the old vials. Populations of around 2,000 adults can be maintained in this way. Smalcova (1970) found that 3,000 eggs were laid on each newly introduced vial containing 5 ml of food. Intense larval

competition is found with such a maintenance system and of the eggs laid only about 9% survived through the pre-adult stage.

In contrast Kinross & Robertson (1969) maintained cages with large amounts of food (350 ml) supplied once a week, each pot of food remaining in the cage for three weeks. These populations maintain about 5,000 adult flies. It was found that the survival of eggs from laying to emergence was highest (c.40%) for eggs laid on the pot in the first two days but had declined almost to zero by the 5th day. It follows that about 10% of all eggs laid will lead to adult flies. Thus, although similar egg mortalities are found under both systems of cage maintenance the most obvious difference lies in the amount of food available to surviving larvae. The 10% surviving in vials undergo severe competition for space and food.

This might go some way to explaining the differences between results of Kearsey & Barnes (1970) and Robertson (1970). Under Robertson's cage conditions little selection pressure may be present at the larval stage, thus no response may be found for his segregating populations.

Which system is more realistic in nature is difficult to say. Conflicting evidence suggest both systems may be equally valid. Sokoloff (1957) found no evidence for variation in weight of wild caught adults in D. pseuodoobscura, D. persimilis, and D. miranda. MacFarquhar & Robertson (1963) found on the other hand measuring body size in D. subobscura very large differences in body sizes in wild flies compared to laboratory reared flies.

From the geneticist's viewpoint it is the differential survival of certain kinds of individuals over others which is important. If

competition does occur in nature, it would be interesting to see if genetically different individuals have the same or different survivals when competition occurs. There are a few cases in which visible mutants have been used to test this proposition. Moree (1952) using the e^{ll} mutant found that the magnitude of natural selection is influenced by competition. As the degree of competition increases, the viability of the e^{ll} mutant is considerably decreased relative to wild type. Moree & King (1961) using e^{ll} found that at all stages of competition the relative viabilities of F_2 formed the sequence $+/e^{ll} > +/+ > e^{ll}/e^{ll}$, the lowest e^{ll}/e^{ll} viability coincided with the highest degree of competition of larvae which they estimated as 90% of the total selective effect. Similar results were found by Dawood & Strickberger (1964) using ebony, but Buri (1956) could detect no selective differences between bw and bw^{75} alleles in population cages. In using major mutants for such studies the problem of overdominance for fitness may obscure the effects of competition on survival, since differences in genetic background are difficult to eliminate completely (Frydenberg, 1964; Polivanov, 1964).

The investigation undertaken by Bakker (1961) revealed that the main differences between competing strains was in larval feeding rates which account for differential survival rates of different combinations of mutant strains. Bentvelzen (1963) comments that the genetic structure of a population is determined in part by the effects of larval density. At high densities a wide range of growth rates would be favourable. He considers investigations along lines more similar to natural conditions than customary population cage methods.

Larval competition may explain the occurrence of different inversions found in D. pseudoobscura at different times of the year. Birch (1955) concluded that selection is a function of density. He found that CH inversions were favoured by low density of larvae and ST inversions were favoured by a high density. Birch suggested this could be analogous to the situation found in the natural habitat where the frequency of CH is high in spring but is then replaced by ST in summer. Recently Druger & Nickerson (1972) could find no differential mortality between AR and CH inversions in populations of D. pseudoobscura maintained under competitive conditions in comparison to non-competitive conditions. Presumably AR and CH have equal viabilities.

From the review of the literature it is evident that competition at the larval stage of Drosophila may be an important factor in selection. When sternopleural chaeta number has been studied under highly competitive larval conditions, evidence has been found for selection acting on this character, (Linney, Barnes & Kearsey, 1971). Conversely under non-competitive larval conditions no selective effects have been found (Robertson, 1967).

The purpose of this study is to test the hypothesis that genes controlling sternopleural chaeta number contribute to larval competitive ability. Such a relationship would imply pleiotropic gene action. If evidence for this hypothesis is found then the nature of the selection can be investigated further, that is to distinguish between the models of optimum and homeostatic selection.

Before the hypothesis can be tested, the environmental effects of larval competition on the individual and the interaction between individuals will have to be quantified. It has been shown that food

shortage at the larval stage affects adult survival, emergence time, adult body size and sternopleural chaeta number (Parsons, 1961; Sang, 1949). Also environmental factors will vary considerably when flies are reared in large numbers as are cage populations under different feeding regimes. Some measure of this variation can be obtained by comparing adult body size in each feeding regime, assuming that larval competition is reflected in an increased variation in adult body size.

A measure of the environmental effect of competition on chaeta number itself can be obtained by rearing flies of the same genotype together over a wide range of chaeta numbers under non-competitive and competitive conditions.

Having dealt with the environmental effects of larval competition, it is possible to investigate whether there is a differential survival among mixtures of genotypes during larval competition. This can be simplified if to begin with single locus systems are used. The frequency of a mutant segregating with its wild allele would be expected to decrease at a faster rate under highly competitive conditions than under non-competitive conditions. This can be tested using several visible mutations.

Turning now to sternopleural chaeta number, it will be more difficult to detect genotypic differences in larval survival in wild populations as the phenotypic range of chaeta number is small. However, artificial populations can be constructed from crosses between selected lines thereby producing very large amounts of variation for chaeta number and selection during the larval stage should be easily detected.

Considering the artificial populations set up by Robertson (1967), if the hypothesis that chaeta genes do contribute to larval competitive ability is true, then rearing these populations under highly competitive conditions should result in a return to the original wild population mean.

As a consequence of constructing artificial populations from extreme selection lines there are problems of background heterozygosity. Any selective effects contributed by chaeta genes may be obscured by heterotic effects from perhaps many other genes fixed by chance during inbreeding in each selection line. It may be that a sub-vital gene has become fixed in one of the selection lines but not in the other line. This can be solved by constructing populations of homozygous lines and an experiment will be described using this technique which is similar in principle to that employed by Linney, Barnes & Kearsey (1971).

The final section will be devoted to examining populations recently isolated from the wild. If chaeta genes have an important effect on fitness through their contribution to larval competitive ability, then the equilibrium values found in wild populations should correspond approximately to maximum fitness values. If these equilibrium values are disturbed by selection, then on relaxation the populations would be expected to return to their original values under competitive conditions.

It is hoped that through these lines of enquiry the disagreements found in published experimental results can be resolved and definite conclusions drawn.

CHAPTER TWO

THE ENVIRONMENTAL EFFECTS OF LARVAL COMPETITION ON THE ADULT POPULATION

Introduction

When the effect of natural selection on a character is being investigated, it is important that the environmental factors also affecting that character are understood. In the case of sternopleural chaeta number in Drosophila it is known that temperature and food shortage affect this character (Thoday, 1958; Parsons, 1961). The character is not affected directly by these two environmental factors but through an association with body size. As adult body size decreases so also does the number of sternopleural chaetae decrease. There is a direct relationship between surface area and chaeta number (Gibson et al, 1961).

The effect of temperature is found to be associated with time of development and final body size. At high temperature (30°C) development time can be reduced to 6 days and consequently body size is reduced. This results in a lower chaeta number score. At low temperature (15°C) development time is extended to around 21 days and body size, and consequently chaeta number, are increased.

Food shortage can be due to either an insufficient amount of food per individual, through changes in nutritive value or drying out of the food source, or it can be due to a relative shortage in terms of competition for the same food source. Both these situations will cause a reduction in adult body size accompanied by a reduction in chaeta number. It is the effect of larval competition on chaeta number, which is the main interest of this thesis. Temperature is not considered and therefore all experiments have been carried out at a constant temperature of 25°C.

Parsons (1961), investigating the effect of larval competition on sternopleural chaeta number, achieved large reductions in body size by using a tyrosine inhibitor, phenyl-thio-carbamide (P.T.C.), as an additive in the food medium. This chemical interferred with growth hormone control and consequently extended the development time and reduced adult body size. His results from a low and a high larval density showed that body size and chaeta number were highly correlated. This relationship appeared to be linear and thus chaeta number is proportional to body size. He also found that while the variation in body size increased with increasing concentrations of P.T.C., the variation of chaeta number remained constant.

Another investigation into the relationship between larval competition and chaeta number was carried out by Kearsey & Barnes (1970). Using standard food medium they set up egg densities in the range from 100 to 1,500 eggs per vial. Although they did not measure body size, the average chaeta score was reduced by three and a half chaetae at the highest density. Very high mortalities in the region of 90% were recorded at the highest density. Their results at the 1,000 density corresponded well with cage conditions in their laboratory. In this chapter it was decided to use the levels of competition similar to Kearsey & Barnes, using standard food medium. It was felt that this would simulate natural conditions more than by adding chemical inhibitors to the food medium.

The purpose of the first experiment was to measure the effect of various levels of competition on development time, body weight over the emergence period, adult survival, and sternopleural chaeta number.

In the second experiment cage populations, which were maintained on

various levels of food supply, were scored for body size and chaeta number. Direct comparisons were made with experiment 1 in an attempt to relate the level of larval competition in the cages to the levels of controlled competition in vials. The reason for making this comparison is that large cage populations will be used in later experiments for the detection of selection. The effects of random fluctuations in gene frequencies can be ignored in large cage populations, and this is an important consideration when investigating selective processes.

In the third experiment the magnitude by which chaeta number is reduced through its association with body size, was investigated. It is important to determine whether this environmental reduction is constant over a wide range of chaeta number phenotypes or whether it changes with increasing chaeta number. Information on this relationship is necessary before any genetic change can be assessed. Since wild populations have a small range of chaeta number scores, possibly because of past selection, it was essential to produce an artificial population possessing a wider range of scores. This was done by using a cross between lines which had originally been selected for high and low chaeta number from a laboratory population.

Experiment 1 The Effects of Larval Competition on Adult Development Time, Body Weight, Survival and Sternopleural Chaeta Number

Materials and Methods

A population of Drosophila melanogaster which had been originally collected in Kaduna in Northern Nigeria about twenty years ago and has been maintained in this laboratory as a large cage population of approximately 5,000 adults, was used.

Eggs were collected from this population by transferring samples of about twenty females from the cage into vials, which contained plastic partitions coated with a mixture of starch and charcoal. The females were allowed to lay for approximately three hours and the appropriate number of eggs transferred into 3" x 1" glass vials containing 5 ml of standard maize meal, yeast extract and molasses food medium. The vials were incubated at 25°C.

The egg samples were set up as follows:-

Density	Replicates
100	30
500	6
1,000	3
1,500	2
3,000	1

The forty-two vials making up the five densities were set up in a random sequence over a period of seven days. As the flies began to emerge, the numbers of each sex were counted and samples of females were weighed each day from each vial. When the majority of the flies had emerged, samples of females were also scored for sternopleural chaeta number.

Results

The results are shown in tables 1 & 2 and in figures 1-3. These confirm the findings of Parsons (1961) and Kearsey & Barnes (1970) in that with increasing density or competition of larvae the time of emergence is extended, and adult weight and survival are reduced. At the highest density the average time of development (table 1) has been delayed by ten days. On average adults took twice as long to develop at high density as compared to low density. There is no difference in developmental time between the sexes, except at the lowest density where the difference is highly significant. However, the difference is small being only 0.2 of a day and it is considered to be no real importance.

As regards adult weight of females (table 1, figure 2) there is a reduction on average of more than a half at the higher densities. The average weight of some adults at the high densities has been reduced to a fifth of maximum weight. As the density increases, the developmental time is extended and although average weight is reduced, at the 500 density it is held fairly constant. At the 1,000 & 1,500 densities developmental time is again extended, but now body weight drops off steeply. It is interesting that at the highest density of 3,000, body weight is maintained at a high level for the first half of the emergence period and then drops steeply.

In figure 3 survival shows a marked decline with increasing density, although as Sang (1949) pointed out numbers are maintained at the expense of developmental time and body weight. However, as the density increases beyond 500 per vial the survival rapidly decreases. As indicated in table 2 there was no sex difference in survival, the

Table 1 Effect of density on emergence time and female weight

Egg Density	Average Developmental Time			Average Female Weight
	♀	♂	t	Weight (mg)
100	8.9500±0.0110	9.1553±0.0100	8.74***	1.2566±0.0381
500	13.5518±0.1309	13.4236±0.1140	0.59NS	0.8504±0.0187
1,000	16.0837±0.1319	16.2398±0.1539	0.55NS	0.5622±0.0652
1,500	15.9557±0.1536	15.6770±0.1466	0.95NS	0.5685±0.0697
3,000	18.7941±0.4949	20.1318±0.4951	1.35NS	0.6972±0.0645

NS = Non significant *** 1% level

Table 2 Effect of density on survival and chaeta score

Egg Density	Replicate No.	Total Nos.		χ_1^2	% Survival	Female Chaeta Scores		
		♀	♂			$\bar{X}$ (150)	V_X	CV(%)
100	29	1221	1133	3.29NS	81.17±1.25	18.0000	2.6979	9.125
500	6	926	871	1.68NS	59.90±5.16	17.0266	2.6435	9.549
1,000	3	585	517	4.20*	36.73±1.39	16.6200	1.9284	8.355
1,500	2	339	322	0.44NS	20.03±8.97	16.0800	1.8727	8.510
3,000	1	102	91	0.63NS	6.43	16.5257(97)	1.9186	8.382

Homogeneity of variance $\chi_4^2 = 9.89$*

* 5% level

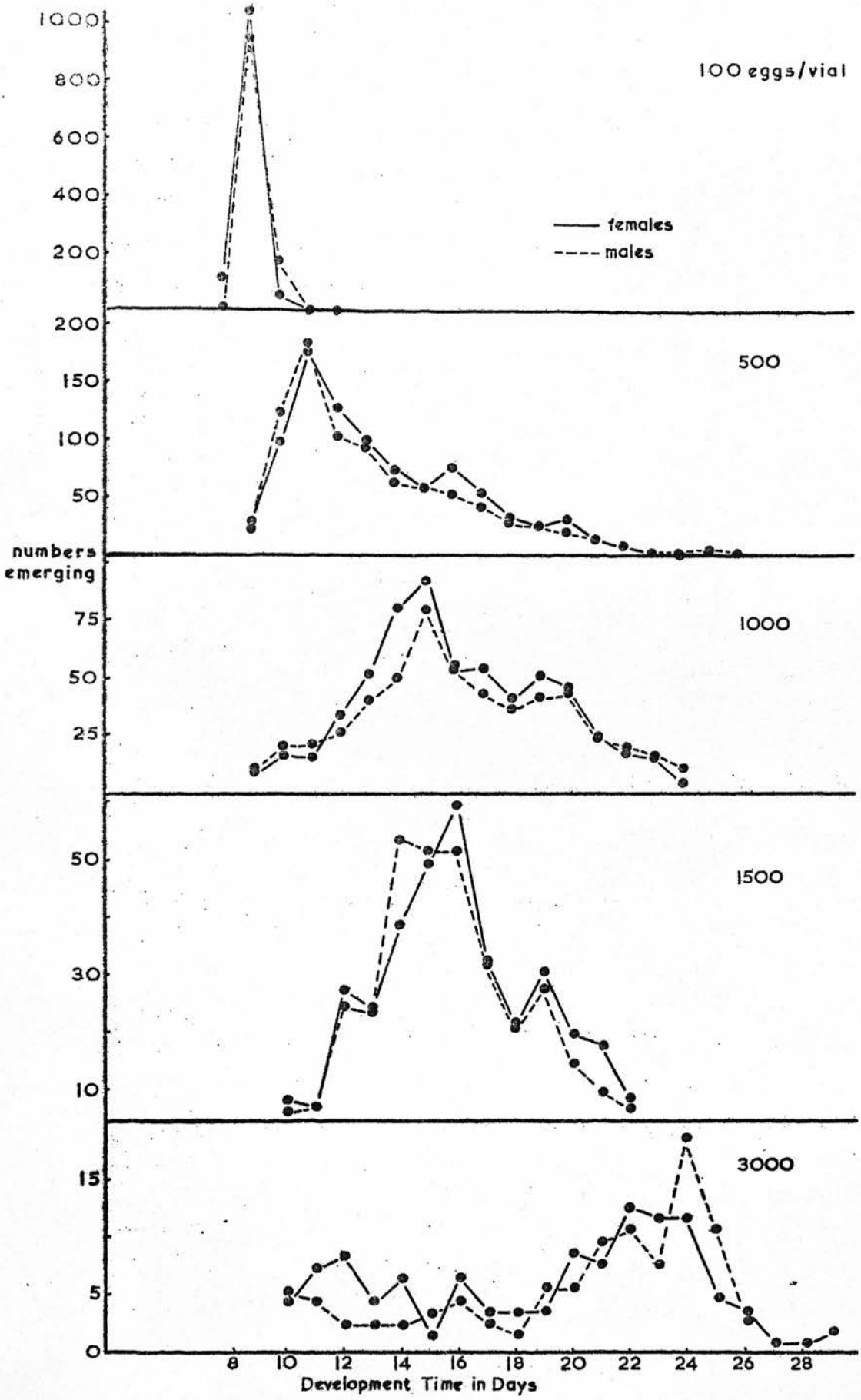


FIGURE 1. THE EFFECT OF DENSITY ON THE RATE OF DEVELOPMENT IN KADUNA.

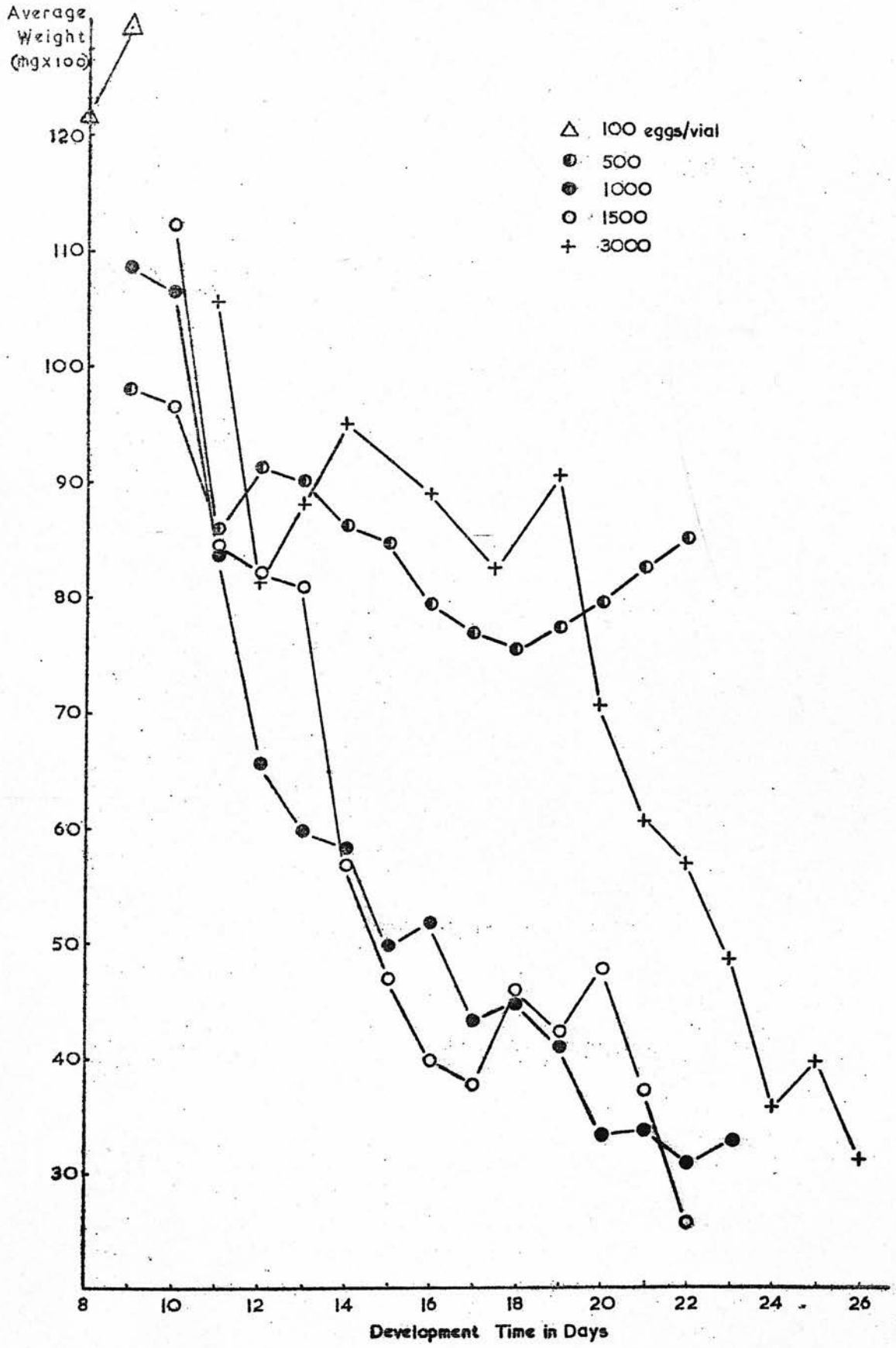


FIGURE 2. THE EFFECT OF LARVAL COMPETITION ON AVERAGE FEMALE WEIGHT.

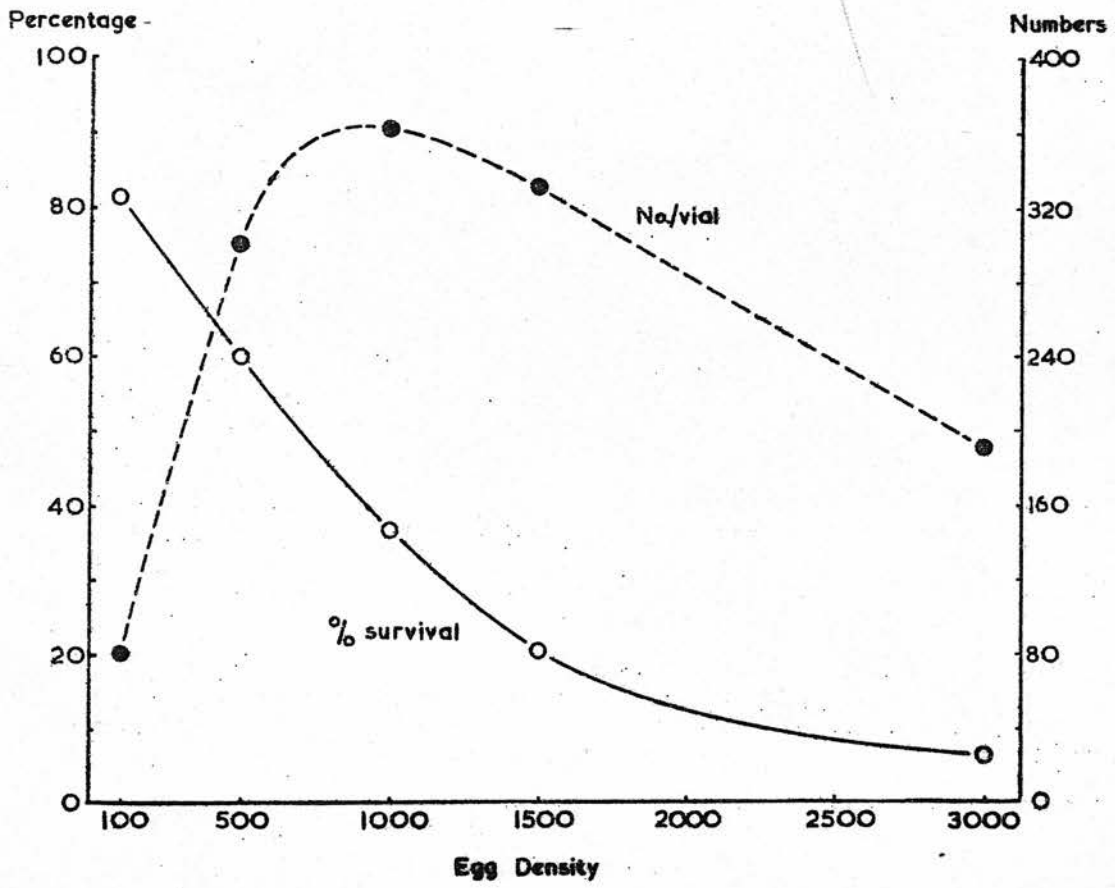


FIGURE 3. THE EFFECT OF LARVAL COMPETITION ON SURVIVAL.

significance at the 1,000 density being marginal. One of the replicates at the 100 density dried out.

In table 2 it can be seen that the average chaeta number score for females decreases with increasing density up to the 1,500 density. At the 3,000 density, however, there has been a slight increase. This increase corresponds with a similar increase in average body weight at this density shown in table 1. These results confirm the relationship found by Parsons (1961). However, there has been a decrease in variation in chaeta number over the densities. As shown in table 2 significant heterogeneity was found among the variance estimates using a Bartlett's test (Snedecor & Cochran, 1968).

Experiment 2 Larval Competition in Kaduna under Different Population Cage Regimes

Materials and Methods

Three population regimes were set up using the Kaduna population. The first regime was that commonly used in this laboratory, in which the population was kept in wooden boxes measuring 32cm x 32cm x 24cm with glass tops. Three holes, 7.5cm in diameter, were cut in the sides for ventilation and a hole, 12.5cm in diameter, was cut, to which a nylon sleeve was attached, allowing access into the cage. Once a week a pot containing 350 ml of standard food medium was placed in the cage. Each pot of food remained in the cage for three weeks. This system maintained a constant population of about 5,000 adult flies (Kinross & Robertson, 1969). The second regime was that used by Barnes (1968), in which the population was maintained on a rotational system of glass food vials. A wooden cage was adapted to this system by boring twenty holes in the base and attaching 3" x 1" glass vials in position. Two vials were attached to the base of the cage on a Monday and Wednesday and four vials on a Friday, the vials being attached at random to the base of the cage. Any one vial would stay attached to the cage for about 18 days. The total amount of food supplied per week was about 40 ml. Approximately 3,000 flies were maintained on this system (Smalcolva, 1970). The third regime was similar to the pot system, but in this case a group of seven vials instead of a pot was placed in the cage each week. Each group of vials remained in the cage for three weeks, so that there were 21 vials present at any one time. The total amount of food supplied by the 7 vials was 35 ml per week. One drop of live brewer's yeast

was added to each vial used in the maintenance of the rotational and 7 vial cages. No live yeast was added to the food in the pot cage.

A small population of Kaduna was also maintained in a half pint milk bottle containing 100 ml of standard food medium. Adults were continuously tipped over into a fresh bottle as they emerged. The old bottle was discarded after three weeks.

The level of larval competition within a cage population is assumed to be reflected by the distribution of adult body size. Body size was assessed by measuring thorax length using the method of Robertson & Reeve (1952). The measurements in this case were not transformed to 3 x log thorax length as it was felt that this transformation would only increase the error of the measurements. Once the populations had built up to large numbers, thorax length was measured on females sampled directly from each population regime and from the bottle culture. Also chaeta number was scored on individuals of both sexes sampled directly from the cages. A control was set up, in which females that had been reared at low density in vials, were scored for thorax length. Also chaeta number was scored on a sample of both sexes from these vials.

In addition two hundred individuals of each sex were weighed from the pot cage and from the rotational vial cage.

Results

In figure 4 it can be seen that a considerable decrease in mean body size, as indicated by thorax length, has taken place owing to the reduction in the food supplied to the cage populations. The rotational vial cage shows the greatest reduction as a consequence of it being

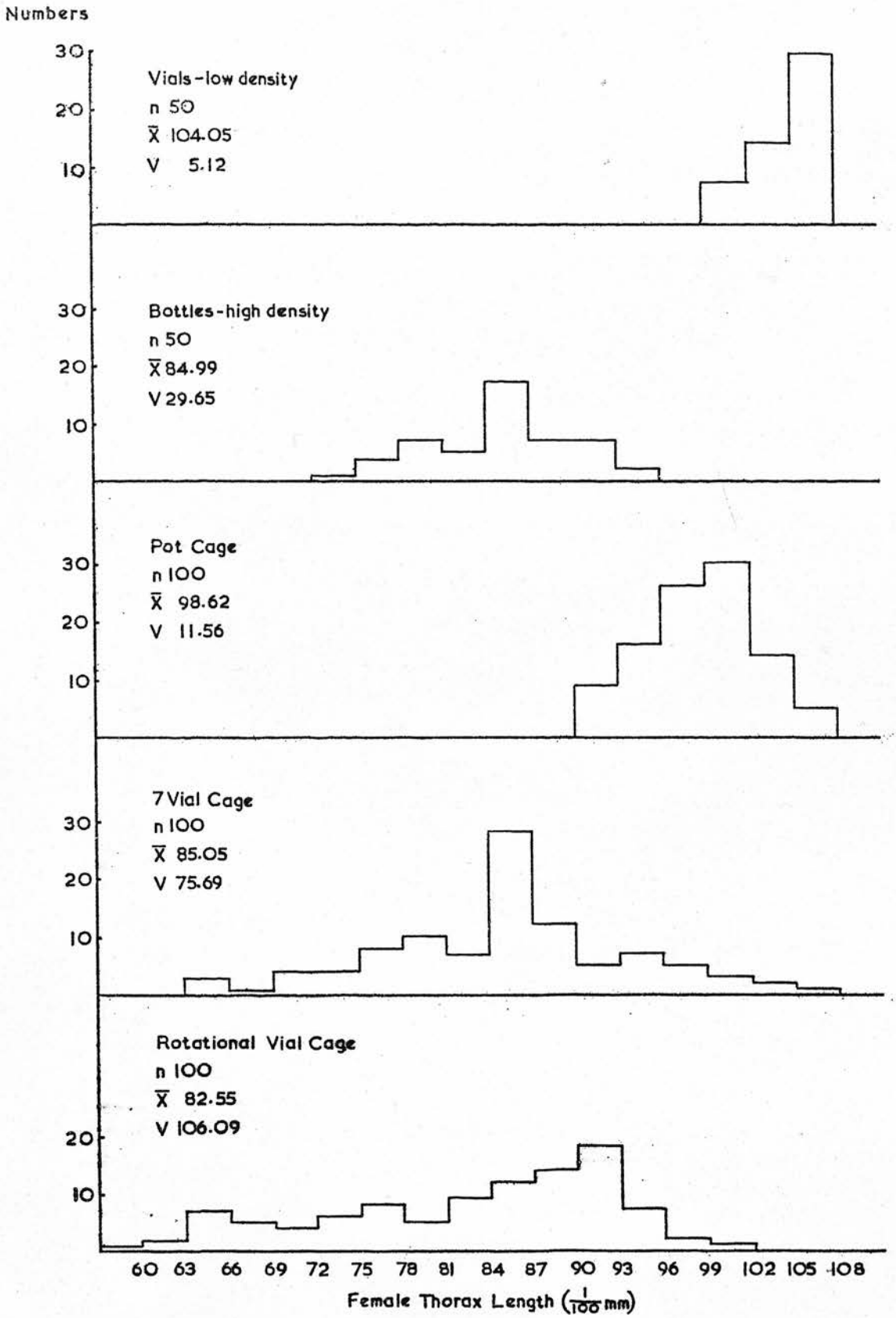


FIGURE 4. THE EFFECT OF LARVAL COMPETITION ON FEMALE BODY SIZE IN THE KADUNA POPULATION.

supplied with small amounts of food at regular intervals. The distribution is skewed in the direction of larger body size. This indicates that a certain number of individuals have maintained a higher body size at the expense of the others, as was found in experiment 1, figure 2 at the 3,000 density. Although the 7 vial cage has the lowest food supply per week, it does not have the lowest mean value. The most interesting result is that of the pot cage, where very little reduction has occurred in comparison with individuals reared under low density conditions in vials. This is in contrast to what had been found previously. Kinross & Robertson (1969) employed a method of estimating several parameters in the Kaduna population maintained on the pot system by the use of marker genes which had been incorporated into the Kaduna background. By introducing numbers of marked eggs into a pot at different times it was possible to measure the survival rates. They reported that about 6,000 adults emerged from a pot and inferred that about 12,000 eggs were laid per day on a pot, but this number declined as larval activity increased. Eggs laid in the first two days had about a 40% chance of survival to emergence but this had declined to zero by the fifth day. They concluded that in order to maintain a stable population size about 10% of all eggs laid would lead to adults. They also report that adult body weight declined over ten days of emergence from a pot from 1 mg to a minimum of 0.5 mg. In the present investigation samples which were taken from the same Kaduna population gave no evidence of a range of individual weights of this magnitude. The mean weight of adults was 0.94 mg and although only females were measured for thorax length, there was no evidence that female weights were much lower than 0.9 mg.

As regards the variation in body size there has been a large increase in both the vial cages, but the variation in the pot cage is small and comparable with that of the low density vials. The bottle population shows a similar pattern to the vial cages with a decrease in the mean and an increase in the variation of body size. This information is of relevance to experiments reported in chapter 5.

The weights of the samples of females and males from the pot cage and the rotational vial cage are as follows:-

	Pot cage	Rotational Vial cage
Females	1.06mg	0.65mg
Males	0.81mg	0.47mg

The female weights correspond well with those in table 1. The pot cage weights are similar to those at low density and the rotational vial cage weights with those at high density. The male weights show the same magnitude of change as those of the females.

As there is a positive correlation between body size and chaeta number (Parsons, 1961), it would be expected that chaeta number would decrease with decreasing body sizes. As indicated by table 3 this relationship is borne out, although the rotational vial cage, which has the lowest average body size, does not have the lowest chaeta score. It is worth noting that the reduction in variation of chaeta number is more marked in this vial cage being almost half that of low density conditions. These results correspond fairly well with chaeta scores under controlled larval competition in table 2 for females.

Table 3 Chaeta scores of three Kaduna population regimes and a control

	N	$\bar{X}$		V_x	
		♀	♂	♀	♂
Low density (vials)	131	18.3435	17.8015	3.0272	4.4218
Pot cage	200	17.8950	17.3550	2.9386	3.6170
7 vial cage	50	16.2600	15.5400	2.8902	2.2127
Rotational vial cage	50	16.8200	16.0200	1.8648	2.0608

Experiment 3 To Measure the Magnitude of Environmental Reduction in Chaeta number

Materials and Methods

The population used in this experiment and in chapter 4 was derived from a cross between a high and a low line, that were originally selected for sternopleural chaeta number from the Kaduna population.

C_3A had been selected for high chaeta number for over twenty years and was assumed to be fixed for almost all increasing chaeta number genes (Robertson, 1967). It had plateaued at about 49 chaetae. DF had been selected for low chaeta number for the same period and had plateaued at about 9 chaetae (Osman, 1963). A population was constructed by Robertson (1967), in which a C_3A third chromosome was allowed to recombine with a DF third chromosome in a C_3A background. Thus the third chromosome was segregating for high and low chaeta number genes in an otherwise homozygous background. This synthetic population had a mean of 28 with a range of 35 chaetae compared to the Kaduna base population, which had a range of 6.

A sample of males from this population, which had been reared at low density, were scored for chaeta number. A representative male of each chaeta score over the range 14-20 and 32-39 was selected. Each male was allowed to inseminate 10 tester females. There were then a total of 15 female groups each having been inseminated by a single male. From each female group eggs were collected and incubated for 24 hours at $25^{o}C$. First instar larvae were then set up at low and high densities in a random sequence for each group of females. The low density was set up at 10 larvae per vial using ten replicate vials. The high density was set up at 50 larvae per vial using 6

replicate vials. Three progeny of each sex from each vial were scored for chaeta number at low density and five progeny of each sex at high density. The total number emerging from each vial and the average weight of males from each mating was recorded.

The tester stock which was used was K_{13} in C, which had a single homozygous third chromosome from Kaduna in a C_3A background. The mean was 31±0.28. As ten females were being used with each male only small numbers of eggs were available and so the density levels had to be scaled down. Small vials measuring $1\frac{1}{2}$" x $\frac{1}{2}$" were used with 1 ml of standard food medium which had been diluted by a half. Also larvae were used in order to avoid differences in hatchability and to minimize age differences.

The reason that extreme scoring males were used in this experiment was because a more accurate estimate of a regression slope can be obtained by using this design of experiment (Hill, 1970). Employing this experimental design can only be justified if the relationship is known to be linear. From a small pilot experiment it was found that the progeny score regressed against the father's score gave a linear relationship.

Results

The analyses of the results are presented in table 4. From the analysis of vial scores it can be seen that there are significant differences between the female groups, as expected, but also there are significant differences between replicate vials within groups. The reason for the replicate differences is that the male parents were segregating for high and low chaeta number genes on the third chromosomes. As there may be original parental high and low chromosomes

Table 4 Experiment 3: Regression analysis of male progeny tests

Low Density

Source of Variation	df	MS	P
Regression	1	7651.2309	< 0.005
Remainder	8	111.4549	< 0.005
Within groups	58	40.4336	< 0.005
Within vials	372	8.2939	

$\hat{b} = 0.4124 \pm 0.0498$

High Density

Source of Variation	df	MS	P
Regression	1	2399.4781	<0.005
Remainder	8	38.8741	<0.005
Within groups	39	32.0706	<0.005
Within vials	435	6.1248	

$\hat{b} = 0.2322 \pm 0.0296$

Joint Regression Analysis (difference between slopes)

Source of Variation	df	MS	P
Overall regression	1	9301.5517	<0.005
Heterogeneity	1	749.1573	0.01-0.005
Remainder	16	75.1645	<0.005
Within groups	1126	8.2998	

segregating within the population which have not been broken down by recombination, it is possible that intermediate scoring males may be the result of a balance between a high and a low parental chromosome. Such a case is found in the progeny scores of a male scoring 32 chaetae at low density. The overall progeny score is 32.5, but the distribution of scores is bimodal, the modes being 26 and 36. This type of male had been carrying both a low and a high scoring chromosome and therefore resulted in heterogeneity of progeny score between replicate vials. It was decided to pool all the progeny scores within each male group and treat them as a single sample from either a low density environment or from a high density environment.

The overall progeny means plotted against their respective male parents are illustrated in figure 5. The slopes of the regression lines are highly significantly different and thus the environmental component of larval competition has a differential effect on chaeta number in that the greater the chaeta number, the larger will be the reduction in chaeta number. In table 4 both the remainder mean squares are highly significant indicating the heterogeneity of progeny scores. It is considered that this significance is unlikely to be due to non-linearity, as a pilot experiment over the range 19-40 gave no evidence of a curvilinear relationship.

The average survival rate at low density was 75%, which is equivalent to about 150 larvae per normal 5 ml of food. At high density the survival rate was 38%, which is equivalent to around 1,000 larvae per 5 ml of food. The weights of males from low and high density were 0.86 mg and 0.60 mg respectively. This reduction in weight of 0.26 mg compares with 0.34 mg which is the difference

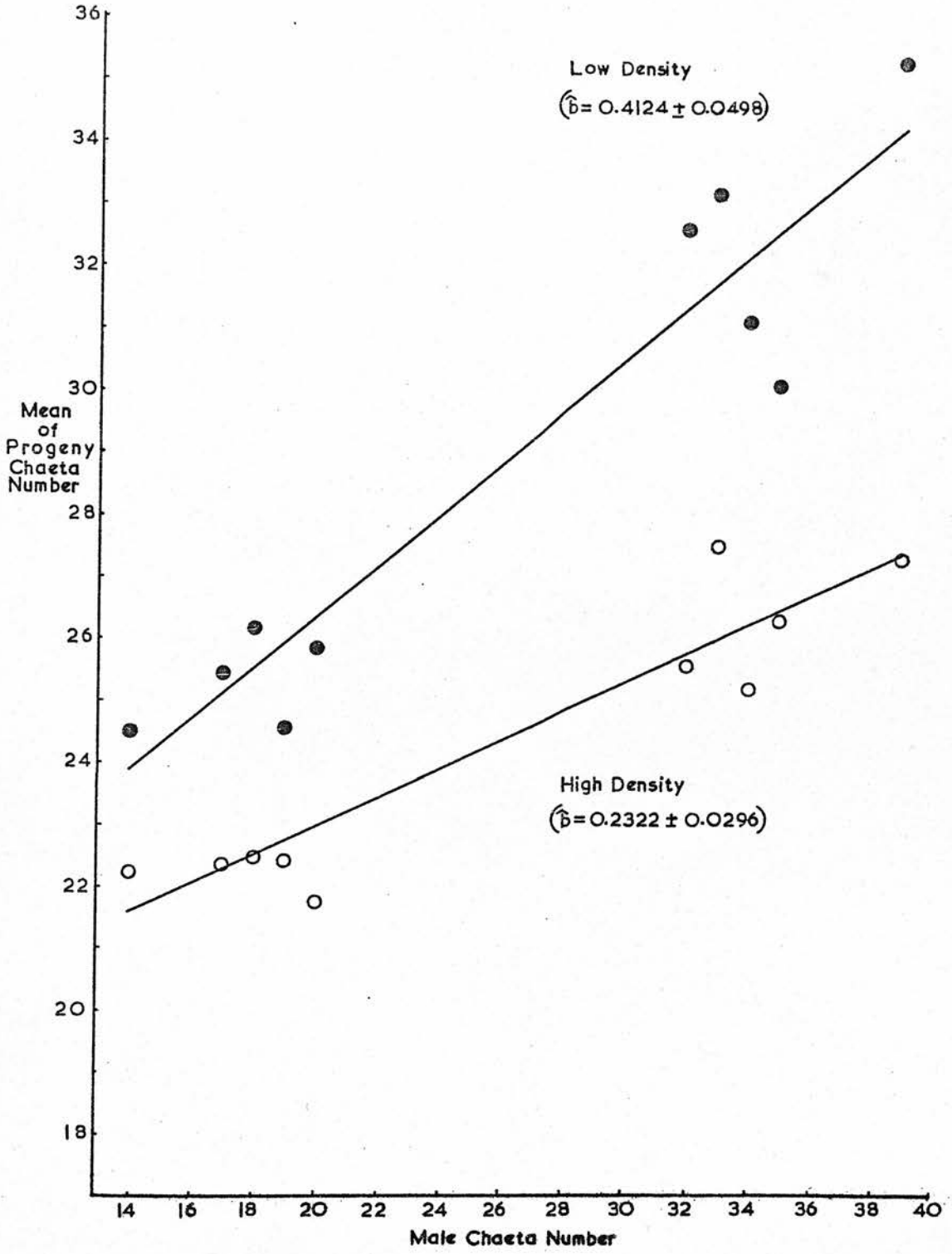


FIGURE 5. THE ENVIRONMENTAL EFFECT OF LARVAL COMPETITION ON CHAETA NUMBER USING SINGLE ca-BLUE MALES.

in male weight between the pot and rotational vial cages. Thus the scaled down experimental design compares favourably with the previous competition trials. It can be seen from figure 5 that the cross between a male parent scoring 14 chaeta and a female from the tester stock produces progeny having a mean score of 24.5 at low density and a mean of 22.2 at high density. Similarly by crossing a male scoring 39 chaeta to a tester female a progeny mean score of 35.2 is obtained at low density, but at high density the mean is 27.1, a reduction of 8 chaetae. Thus over the range of phenotypes, 24-35 the average differential reduction has increased by a factor of three.

Discussion

There are three interesting results which have emerged from the three experiments described.

One is the result from the highest density (3,000) set up in experiment 1. It is evident from figures 1 and 2 that at this density about a third of the individuals which emerged earlier, had a higher body weight than the majority. This is explained by the occurrence of small differences in the feeding rate of larvae and also small differences in the age of eggs, as technically it was difficult to collect 3,000 eggs of exactly the same age. The combination of these small differences could give individuals sufficient advantage in development to maintain a higher body weight at the expense of the other individuals. As there is over 90% mortality at this density, it is likely that the majority of eggs will die through the activity of the individuals hatching first. As the growth of larvae continues, the competition for food becomes more intense and

many individuals will starve.

In contrast to the conclusion of Parsons (1961), that the variation in chaeta number remained constant while body size variation increased at high levels of competition, the variance of chaeta number found here has changed. This could be explained as an effect of scale, as both the mean and the variance have decreased. However, if the coefficients of variation are calculated, it is evident from table 2 that there has been a decrease in the variance over densities. Could it be that competition of this intensity produces the effect of eliminating extreme chaeta number phenotypes? From their investigation Kearsey & Barnes (1970) proposed that the elimination of extreme chaeta number phenotypes did occur through intense larval competition.

The second interesting result is that in figure 4, where the difference in mean body size is very marked between the pot cage and the rotational vial cage. This confirms the supposition put forward in chapter one, that a population which is maintained on a pot system, undergoes little larval competition, assuming that thorax length of the adult reflects the conditions of larval development. On the other hand the distribution of thorax length in the rotational vial cage is very large and reflects intense competition corresponding well with the pattern of results for the 3,000 density in figures 1 & 2. It is probable that this difference in larval competition is not due solely to the difference in the quantity of food provided, but to the difference in the surface area of food available to the surviving larvae. In the pot cage the food dries out slightly and separates from the sides of the pot and thus a large tapering cylinder of food is made available. The same process takes place in the vial cages,

but the surface area is about seven times smaller than the pot cage. Since the bottle culture is a closed system, the food does not contract through evaporation and therefore feeding is confined to the top layer of food, although under extreme competition the larvae may form cavities below the surface of the food.

The chaeta number scores from the three populations in table 3 again follow the same pattern as found in the competition trials in experiment 1, table 2. As with the 3,000 density the rotational vial cage gives the same pattern of reduction in variation in chaeta number. This confirms the disagreement with Parson's conclusion found in experiment 1. This gives further evidence that selection may be operating on chaeta number.

The third result of interest is the differential reduction of chaeta number over the range 24-35 chaetae. The magnitude of the difference in the environmental depression over this range increases by a factor of three. Kearsey & Barnes (1970) assumed in their paper a constant environmental depression of two chaetae over their range of 15-35 chaetae. Clearly this assumption is wrong. The implication of this to their model of selection will be discussed later.

This relationship between chaeta number and environmental depression will be useful in distinguishing between the magnitude of environmental effects and those effects due to selection under intense larval competition.

Conclusions

1. Only at high levels of competition in vials is the variation in body size increased. Although there is a positive correlation of chaeta number with body size, the variation in chaeta number is reduced under highly competitive conditions. Similar results are found in populations undergoing severe larval competition.

2. It is found that larval competition varies between cage populations and is dependent of the amount of food supplied and the frequency of feeding. Thus larval competition is most intense when small amounts of food are supplied frequently. In contrast the level of larval competition is low in populations supplied with large quantities of food infrequently.

3. The amount by which the individual's chaeta number score is depressed through reduction in body size is found to differ over the range of scores. The higher the chaeta number score at low density of individuals, the greater the reduction at high density.

CHAPTER THREE

EXAMINATION OF DENSITY DEPENDENT SELECTION USING VISIBLE MUTATIONS

Introduction

To substantiate the hypothesis put forward in this thesis, it is necessary to demonstrate that the phenomenon of density dependent selection operates at the larval stage in *Drosophila*. Density dependent selection occurs when there is a differential survival among individuals as a result of competition for a single resource. On this model of selection, gene frequency changes are dependent on the density of competing individuals. Instead of using the quantitative character, chaeta number, several visible mutations will be used in this chapter. This can be justified because the effect of density dependent selection should be more easily detected. If a simple case is considered in which a mutant gene(*a*) is recessive to its wild allele(*A*), the following fitness values can be assigned to the segregating genotypes:-

Genotypes	AA	Aa	aa
Frequencies	p^2	2pq	q^2
Fitness	1	1	1-s

Under the model of density dependent selection, the selective coefficient (s) will increase with increasing density of individuals. As the value of s approaches 1.0 under very severe competition, the *a* gene will behave as a recessive lethal.

This type of selection, involving recessive mutants, has been described by Moree & King (1961) and Dawood & Strickberger (1964). In both cases investigations were run over a single generation at several larval densities. Birch (1955) attributed the maintenance of

an inversion polymorphism in Drosophila pseudoobscura to density dependent selection. This investigation was carried out by maintaining populations in bottles for many generations.

On the other hand Lewontin (1955) found that the viability of the recessive white gene varied when competed individually against a large number of other different genotypes. He concluded that the result of competition between any two genotypes could not be predicted on the basis of their own individual viabilities. Other investigators (Buri, 1956; Reed & Reed, 1950; and Merrell & Underhill, 1956) have been unable to detect the effect of density on recessive mutants.

However the results from chapter 2 indicate that cage populations supplied with large quantities of food at weekly intervals do not necessarily create high levels of larval competition. This may be one of the reasons why density dependent selection has not been detected in some investigations.

In this chapter, the survival of four recessive mutations will be examined during competition with their respective wild type alleles at low and high larval densities. The experiments will be run in vials over several generations and in population cages. Egg to adult viability will be measured over a single generation for each of the genotypes:- AA, Aa, aa for the four mutants.

Experiment 4 The effect of larval density on three mutants - scarlet, claret and ebony-sooty

Materials and Methods

Two eye colour mutants, scarlet and claret, and a body colour mutant, ebony-sooty, were used in this experiment. These three mutants are situated on the third chromosome in the following positions:- st-44.0, e^s-69.5, ca-100.7. Scarlet and claret are completely recessive to their wild type allele whereas the heterozygote of ebony-sooty can be distinguished from the wild type but with some difficulty. This distinction was not relied upon for identification of heterozygotes in any part of the experiment.

The stocks used in this experiment had been constructed by Professor A. Robertson and were designated st-D_3inC, ca-D_3inC, and e^s-D_3inC. The background of these stocks was assumed to be homozygous and the third chromosome had come from the DF low chaeta line and the 1st, 2nd and 4th chromosomes had come from the C_3A high chaeta line. (Each of the mutants was incorporated into a D_3inC stock by continual backcrossing and selecting down for chaeta number). The fact that the stocks are constructed from selected chaeta lines is incidental to the purpose of the experiment. It is important for this experiment that each stock should be segregating at one major locus in an otherwise homozygous background. Each of the mutant stocks was crossed to the D_3inC stock carrying the respective wild allele for each mutant, thus producing the three genotypes:- A/A, A/a, and a/a. The purpose of the first experiment was to test whether there is a differential survival among these three genotypes at high larval density as compared to low density. This experiment was run in 3" x 1" glass vials

containing 5ml of standard food medium. Because large numbers of replicate vials were involved in this experiment, it was not possible to use exact numbers of eggs and so density levels were set according to the number of pairs of adults used and the time allowed for egg laying. The low density consisted of four replicates each of twenty vials with ten pairs of adults per vial. The females were allowed to lay for three days and then they were discarded. In every generation the progeny from each of the twenty vials were pooled within each replicate and ten pairs of adults per vial were set up at random in a further twenty vials. This was continued for five generations.

The high density was set up using four replicates each consisting of four vials with fifty pairs of adults per vial. The parents were discarded after seven or eight days. Every generation the progeny from the four vials were collected over a seven to ten day period and were pooled within each replicate and another four vials were set up with fifty pairs of adults per vial. At this density the number of emerging adults fluctuated considerably between replicates and sufficient were often not available for the next generation. In these cases the total number of progeny were split into four equal groups and transferred to four new vials.

Approximately fifty females were sampled per replicate and the proportion of mutants was recorded. The size of the sample varied depending on the number available per generation. From the females sampled at both densities twelve non-mutant females were crossed to the mutant stock to ascertain the proportion of heterozygotes in the sample. In the final generation samples of males were also taken and twelve non-mutants were progeny tested. In order to avoid a bias in the high

density replicate lines when insufficient females were available to set up the next generation a proportionate number of the mutant females were also removed with the twelve non-mutant females.

As a comparison, two population cages were set up for each mutant segregation. A low density cage was set up on pots and a high density cage was set up on rotational vials as described in experiment 2. The discarded parents from F_2 and extra progeny of the F_3 low density generation were used to set up a population cage maintained on pots. Parents of F_3 and extra progeny of the F_4 high density generation were used to set up a rotational vial cage. These cages were run for five months which corresponds to roughly nine generations although the generation time may have little meaning in the rotational vial cage system. Approximately 200 individuals of each sex were sampled after five months and 50 non-mutants of each sex were progeny tested using their respective mutant stock.

Results

The gene frequency estimates of the mutants scarlet, claret and ebony-sooty over six generations are illustrated in figures 6 & 7. From these graphs it can be seen that there are large fluctuations between replicates, although the claret lines are reasonably consistent between replicates particularly at high density. The variation between replicates can be attributed to sampling or genetic drift owing to the small populations used. With each mutant there had been a gradual decrease in gene frequency over the six generations. However it is the estimates in the final generation which are of interest. In this last generation both sexes were progeny tested and gene frequency

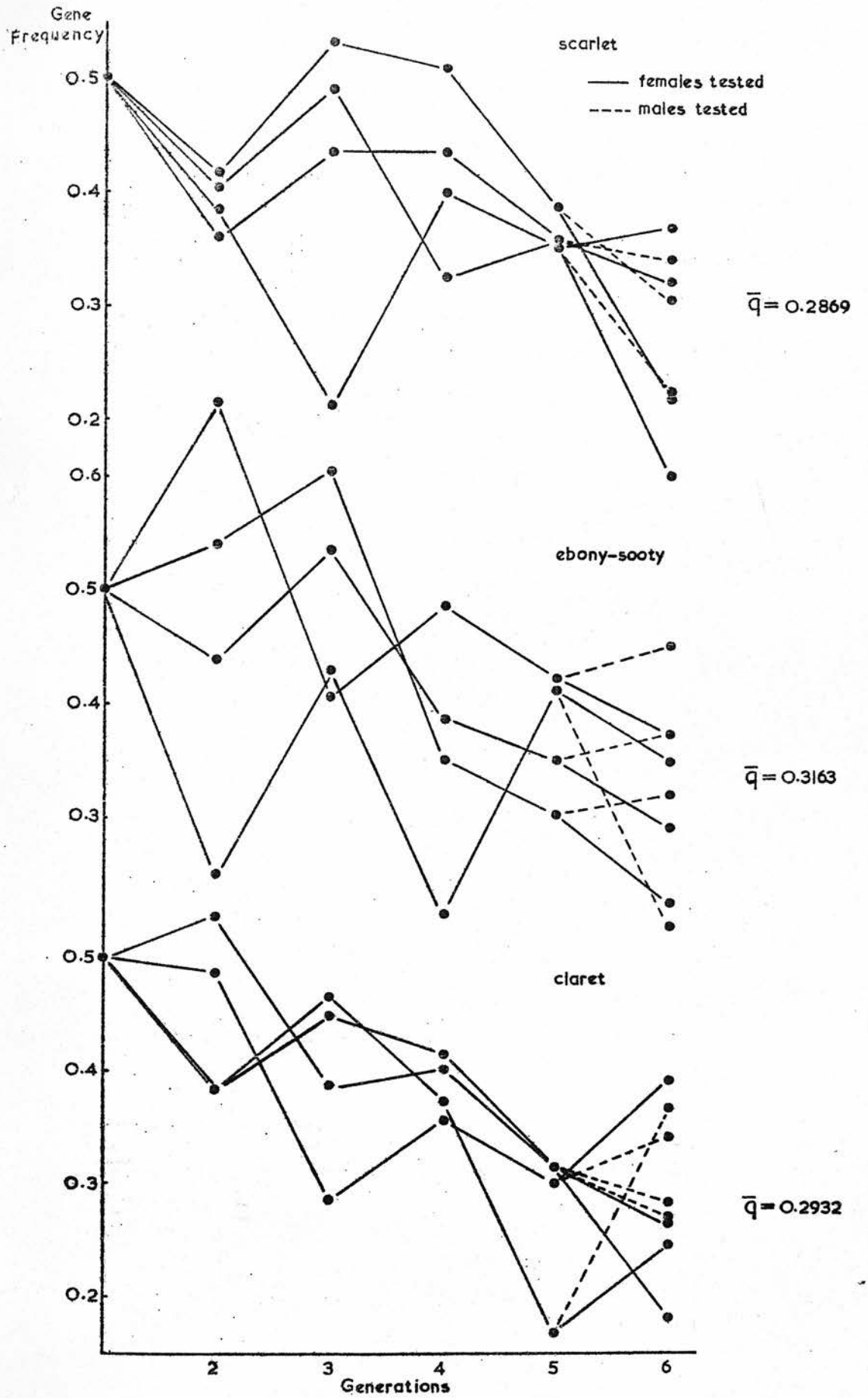


FIGURE 6. GENE FREQUENCIES OF SCARLET, EBONY-SOOTY AND CLARET OVER FIVE GENERATIONS AT LOW DENSITY.

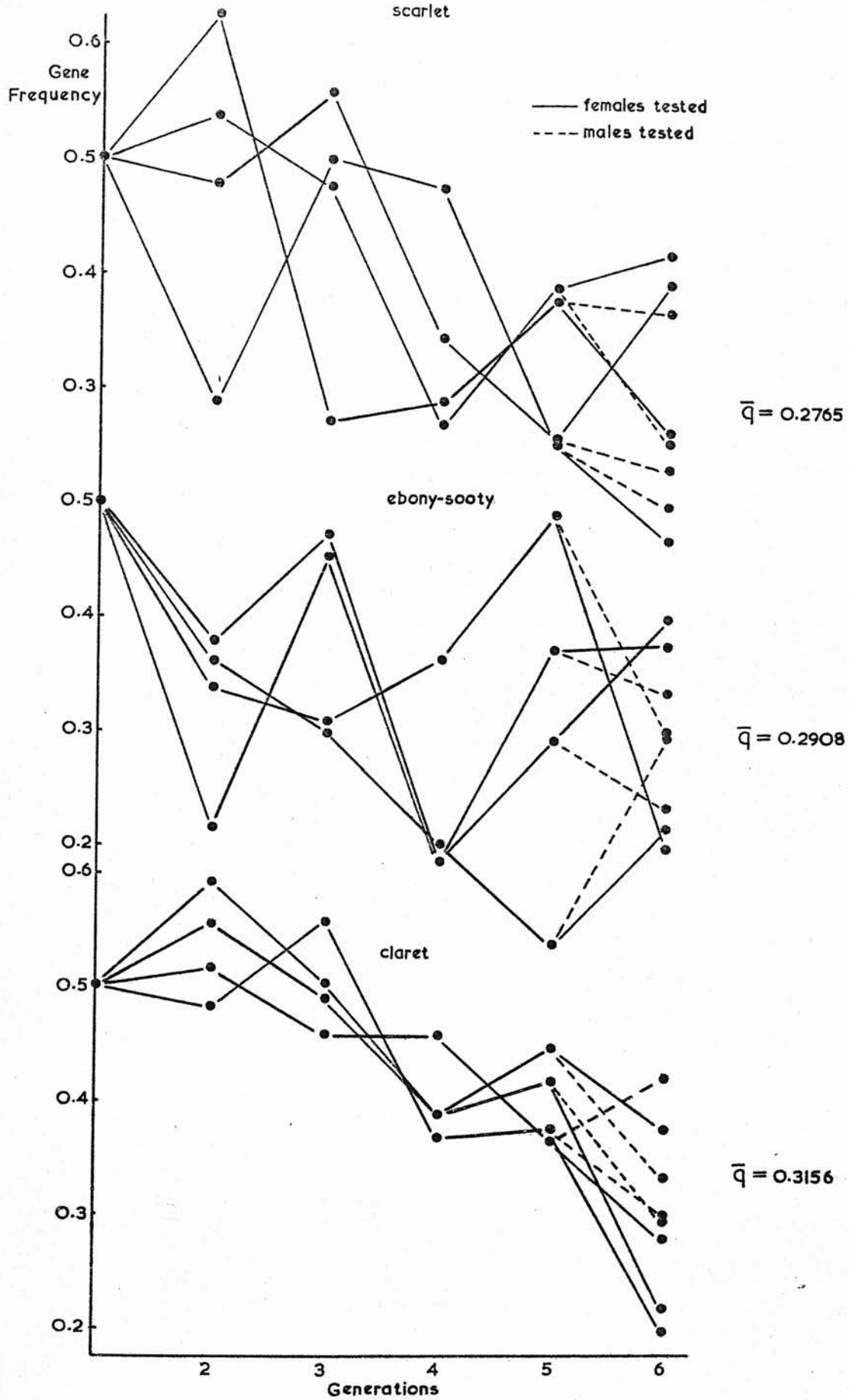


FIGURE 7. GENE FREQUENCIES OF SCARLET, EBONY-SOOTY AND CLARET OVER FIVE GENERATIONS AT HIGH DENSITY.

estimates were obtained for each sex. These estimates are shown in table 5 together with the analysis for each mutant. In every case there is no difference between the density levels or between the estimates from each sex. Intense larval competition has had no effect on the survival of the mutant genotypes. The expected change in gene frequency over six generations has been calculated for low and for high density conditions. At the low density, there may be some disadvantage to the mutant phenotype as compared to the wild type phenotype, as for instance there may be a disadvantage during mating. From experience with class experiments using eye colour mutants selective coefficients of about 0.4 have been found. It has been assumed that at high larval densities the mutant phenotype will not survive and it has been assigned a selective coefficient of 1.0 and therefore a fitness of zero. The expected frequencies are illustrated in figure 8. After six generations of low density conditions the gene frequency of the mutant would have decreased to 0.2925. In contrast the gene frequency at high density would be 0.1429. The observed frequencies over the six generations, averaged over replicates, are shown in table 6 and illustrated in figure 8. It can be seen that the observations fit fairly well with the model of non-competitive conditions. The observations suggest that a selective coefficient of less than 0.4 would be reasonable, somewhere in the region of 0.35. The ebony-sooty frequencies at high density might indicate an effect of competition although there are large fluctuations in frequency over the six generations. The difference between the low and high densities is consistent in every generation. The replicate mean is higher for every generation at low density than the corresponding replicate

Table 5 Analyses of gene frequencies in the 6th generation

Scarlet

	Density	
	Low	High
♀	0.3703	0.2632
	0.1489	0.4178
	0.2196	0.1676
	0.3212	0.3908
♂	0.2232	0.3657
	0.3420	0.2537
	0.3056	0.1980
	0.3438	0.2302

Item	df	MS	VR
Densities (D)	1	0.00001	<1
Sexes (S)	1	0.00086	<1
D x S	1	0.00750	<1
Between replicates	12	0.00799	
Total	15		

Claret

	Density	
	Low	High
♀	0.3948	0.2788
	0.2650	0.3432
	0.2055	0.2981
	0.2452	0.3669
♂	0.3425	0.4200
	0.2570	0.3781
	0.2838	0.1438
	0.3681	0.3268

Item	df	MS	VR
Densities (D)	1	0.00096	<1
Sexes (S)	1	0.00240	<1
D x S	1	0.00154	<1
Between replicates	12	0.00640	
Total	15		

Ebony-sooty

	Density	
	Low	High
♀	0.2227	0.3958
	0.3713	0.1974
	0.3462	0.2163
	0.2895	0.3757
♂	0.3189	0.2344
	0.4535	0.3007
	0.2024	0.2963
	0.3748	0.3329

Item	df	MS	VR
Densities (D)	1	0.0006	<1
Sexes (S)	1	0.0033	<1
D x S	1	0.0013	<1
Between replicates	12	0.0070	
Total	15		

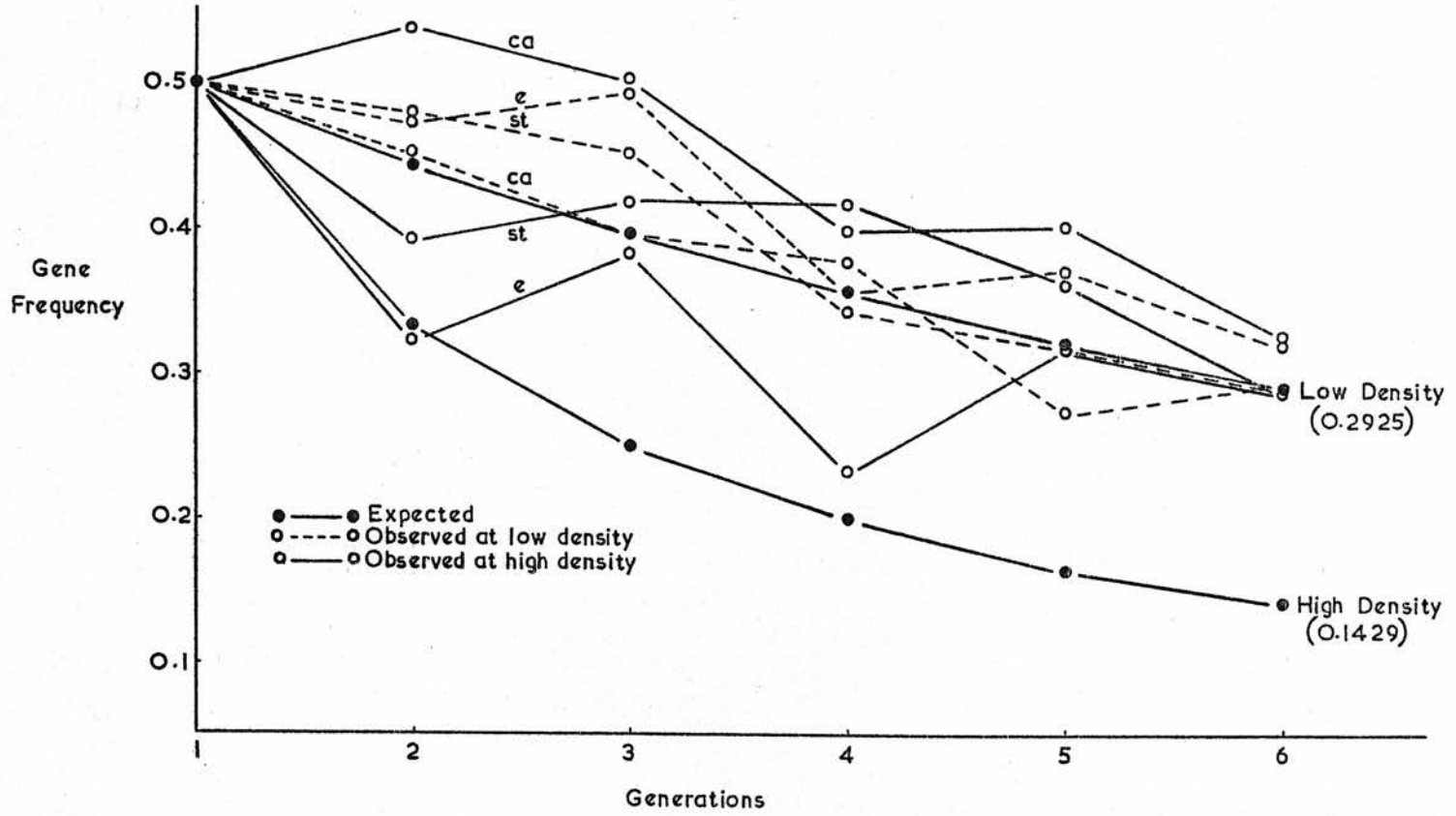


FIGURE 8. EXPECTED AND OBSERVED CHANGES IN GENE FREQUENCIES OF SCARLET, EBONY-SOOTY AND CLARET IN VIALS.

Table 6 Replicate means over six generations

Low Density

Generation	scarlet	ebony-sooty	claret
	Average frequency		
1	0.5000	0.5000	0.5000
2	0.3929	0.4729	0.4471
3	0.4195	0.4941	0.3980
4	0.4175	0.3570	0.3772
5	0.3625	0.3720	0.2751
6	0.2765	0.3163	0.2932

High Density

Generation	scarlet	ebony-sooty	claret
	Average frequency		
1	0.5000	0.5000	0.5000
2	0.4800	0.3233	0.5387
3	0.4520	0.3830	0.5022
4	0.3435	0.2325	0.3998
5	0.3178	0.3168	0.4025
6	0.2859	0.2908	0.3156

mean at high density. However the final generation estimates of ebony-sooty do fit with the expected gene frequency under non-competitive conditions.

The gene frequency estimates from the cage populations are shown in table 7. The estimates have been tested for homogeneity by a 2 x 4 chi-square. The homogeneity chi-square for each mutant is shown in table 7 and indicates that scarlet and claret are similar in frequency for both population cage regimes. The difference between the ebony-sooty cages is highly significant.

The expected changes in gene frequency over twelve generations are shown in figure 9. Under low density conditions the frequency at generation twelve would be 0.1840 and under high density conditions the frequency would be 0.0767. It can be seen that the claret frequency after five months under low and high population cage conditions has behaved in a similar fashion as before in the vial experiment. The scarlet frequencies, although similar, are much lower than expected from the model.

In contrast the ebony-sooty frequencies fit with the model of density dependent selection although the final estimates are lower than expected. However it is the difference between the estimates which is important. The final frequency in the rotational vial cage (high density) is much lower than the final frequency in the pot cage (low density).

It was decided to check the population cages for contamination at the end of the experiment. There are four possible checks on these populations for loci controlling sternopleural chaeta number, female abdominal pigmentation (FAP), alcohol dehydrogenase (ADH) and esterase-6

Table 7 Gene frequencies in the population cages at approximately F_{12}

	Pot Cage				Vial Cage				Homogeneity chi-square
				N				N	
scarlet	$\hat{q}$	♀	0.0563	175	$\hat{q}$	♀	0.0588	207	2.34
		♂	0.0656	179		♂	0.0331	203	P>0.5
ebony-sooty	$\hat{q}$	♀	0.1507	240	$\hat{q}$	♀	0.0133	217	38.49
		♂	0.1534	252		♂	0.0476	162	P<0.005
claret	$\hat{q}$	♀	0.2258	198	$\hat{q}$	♀	0.1744	121	1.51
		♂	0.2065	225		♂	0.1856	129	P>0.75

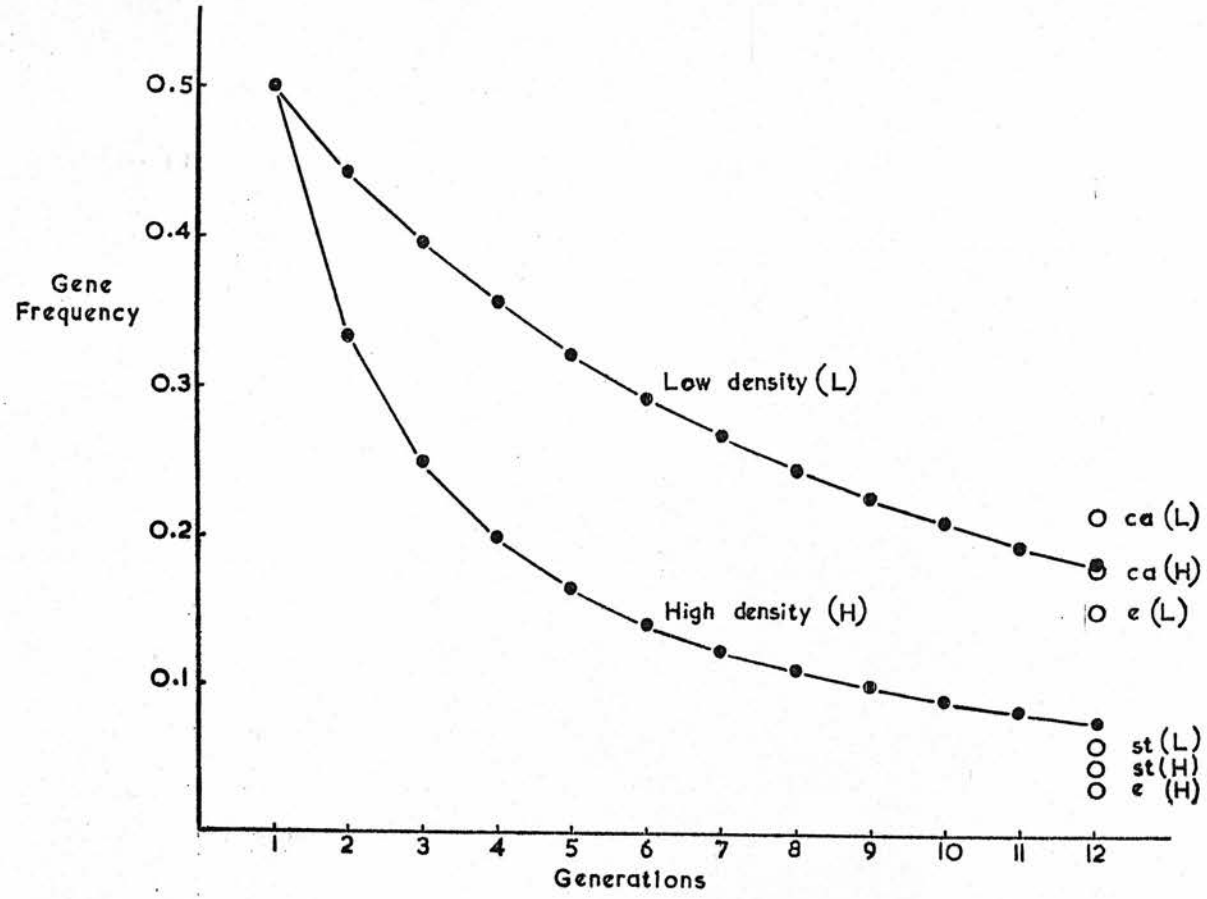


FIGURE 9. EXPECTED AND OBSERVED CHANGES IN GENE FREQUENCIES OF SCARLET, EBONY-SOOTY AND CLARET IN POPULATION CAGES.

(Est-6). The chaeta number score of the D_3inC stock is 15.92±0.22 and all the mutant populations should have similar means to this. By chance the low chaeta line, DF, has become fixed, during selection, for the spot allele (spt) controlling abdominal pigmentation in females (Robertson & Louw, 1966), also for the slow allele of ADH, and for the slow allele of Est-6. The high, C_3A, has become fixed for the other alternatives, light (lt) allele and fast alleles for ADH and Est-6. Thus all the populations should be fixed for abdominal spot, Est-6 slow on the third chromosome and fixed for ADH fast on the second chromosome. The results of all these checks on the populations together with the original stocks are summarized in table 8. Since there is background segregation in both the population cages of scarlet and ebony-sooty, it is likely that the individuals used to establish the cages were segregating for these loci. The scarlet stock, st-D_3inC, used to set up the experiment is segregating for both ADH and Est-6 and this explains the segregation in the cage populations. It must be assumed that the ebony-sooty stock became contaminated early in the vial experiment or that both population cages have become contaminated separately. At least the claret population is consistent throughout the entire experiment.

Checks were not carried out on the vial lines in the first part of this experiment as these lines had been terminated before it was realized that these internal checks were available.

Table 8 Summary of checks on marker loci in experimental populations and mutant stocks

Cage populations

		Chaeta Number	FAP	ADH	Est-6
scarlet	Pot	15.21±0.28	segregating (seg)	seg	seg
	vial	17.92±0.43	seg	seg	seg
ebony-sooty	Pot	17.45±0.43	seg	seg	seg
	vial	16.38±0.37	seg	seg	seg
claret	Pot	15.96±0.31	fixed (spt)	fixed (F)	fixed (S)
	vial	16.17±0.28	fixed (spt)	fixed (F)	fixed (S)

Stocks

st-D_3inC	———	seg	seg
e-D_3inC	16.19±0.32	fixed (F)	fixed (S)
ca-D_3inC	16.10±0.47	fixed (F)	fixed (S)
+-D_3inC	15.92±0.45	fixed (F)	fixed (S)

F = fast S = slow

Experiment 5 Egg to adult viability of the mutants - st, ca, and e^s

Materials and Methods

In this experiment egg to adult viability was assessed for each of the genotypes under competitive conditions using the stocks:- st-D_3inC, ca-D_3inC, e^s-D_3inC, +-D_3inC and their F_1 crosses. Densities were set up using 100 eggs per vial in 1ml of standard food medium in 2" x 3/4" vials. Five replicates were set up for each genotype. The number of surviving adults was noted from each vial.

Results

The results of egg to adult viability are shown in figure 10. Both scarlet and claret show a superiority of their F_1 crosses over both the wild and mutant genotypes, although in the case of claret the superiority is only marginal. There is a sex difference in the progeny from the F_1 between ebony-sooty and the wild stock, the value of chi-square was 6.08 with a probability of less than 0.025. The results for the ebony-sooty have therefore been shown for each sex separately. F_1 superiority is shown for females but not for males. In this case there is a very low survival rate of the mutant genotype. This may be explained by the fact that three of the five replicates dried out in varying degrees during the experiment.

The data from the first part of experiment 4 can be compared with the results from this present experiment. Information on the first generation of segregation has been extracted from experiment 4 in which the three genotypes are competing together. The F_2 segregation ratios have been checked for deviations as a three to one ratio of wild to

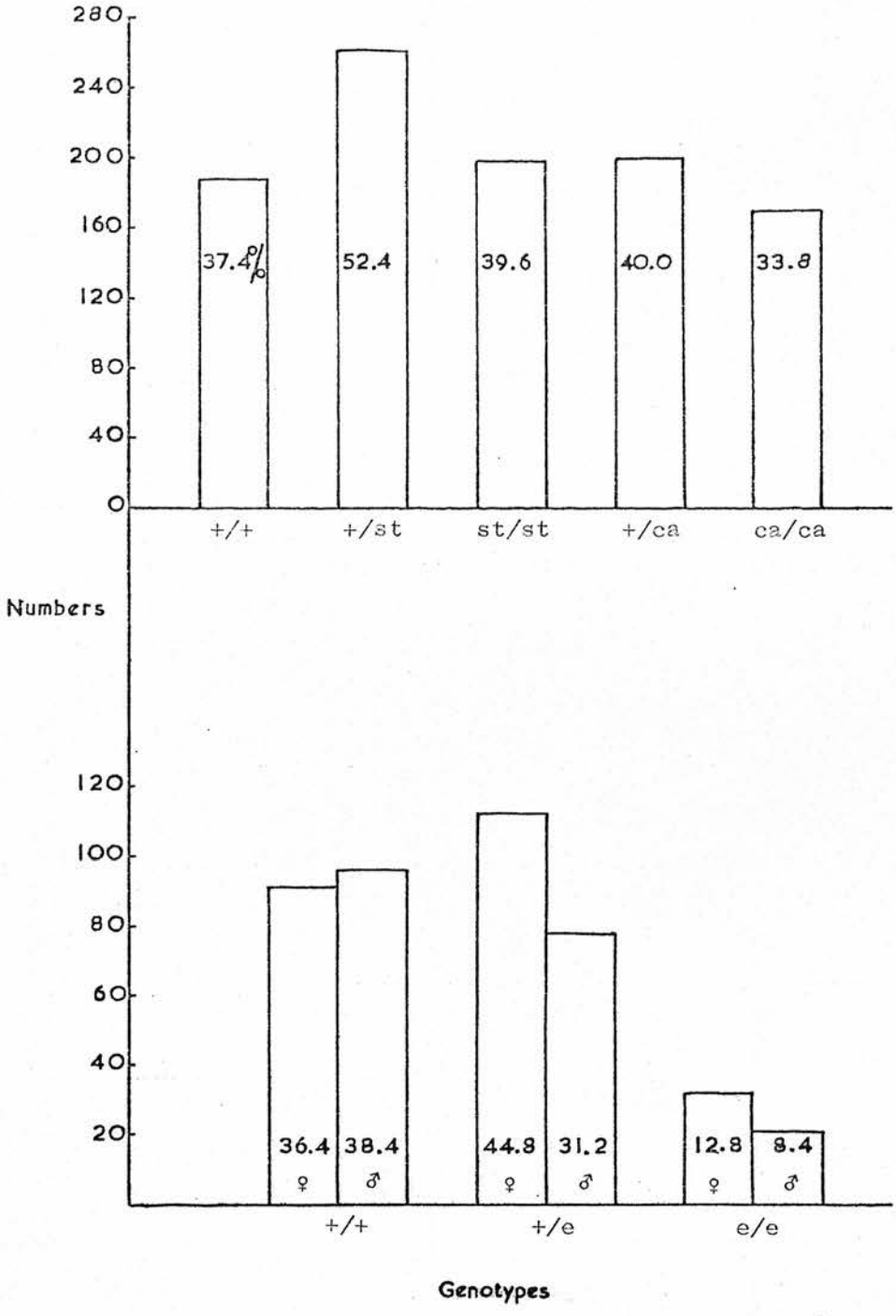


FIGURE 10. SURVIVAL RATES OF SCARLET, CLARET AND EBONY SOOTY AND THEIR F_1 CROSSES.

mutant is expected. It must be remembered that in this experiment, the levels of competition were related to the number of pairs of adult flies per vial and not an exact number of eggs. All the replicates at both densities have been pooled and the results are shown in table 9. The high density conditions have had no effect on the survival of the mutants, scarlet and claret. The ebony-sooty does show a significant deviation from expected, as the mutant genotype has not survived as well at this density. However when the low density ratios are examined the same pattern emerges. Again the ebony-sooty shows a large deviation from expected.

Table 9 F_2 segregation data from experiment 4 carried out in vials

High Density

		Wild type phenotype	Mutant phenotype	Chi-square value
scarlet	observed	71	22	
	expected	69.75	23.25	0.0896^{NS}
claret	observed	119	38	
	expected	117.75	39.25	0.0531^{NS}
ebony-sooty	observed	84	10	
	expected	70.5	23.5	10.3404***

Low Density

scarlet	observed	139	37	
	expected	132	44	1.4848^{NS}
claret	observed	147	38	
	expected	138.75	46.25	1.9622^{NS}
ebony-sooty	observed	112	13	
	expected	93.75	31.25	14.2100***

NS non-significant

*** 1% level of significance

Experiment 6 Survival of a chaeta mutant in competition with its wild type allele

Materials and Methods

The sex-linked mutant, singed, appeared as a rare recessive in a population which had been recently caught in Spain. The mutant was picked up in a control line within a selection experiment which will be described in chapter 5. Contamination can be ruled out since all laboratory stocks carrying this mutant are multiply-marked and no other mutants were detected in this line at any time. This is an ideal system for testing the hypothesis of differential larval survival during the competitive process because no foreign material has been introduced into the background. Also since this is a sex-linked mutant, it can be easily followed within a population by observing the frequency of mutant males. A homozygous singed and homozygous wild line were established from the experimental control line. A cross between homozygous wild females and singed males from the derived lines was set up and maintained as a bottle culture at low density i.e. the parents were discarded after three days. Another bottle was set up with the discarded parents and these flies were allowed to lay eggs for up to eight days before being discarded. The frequency of singed males was recorded for seven generations at low density, but in the high density bottle the males were recorded from generation 4-6 only.

Competition trials were also set up using both reciprocal crosses between the singed and wild lines. Low, intermediate and high densities were set up using 50, 100, and 200 larvae per vial respectively. The vials used were 2" x 3/4" containing 1ml of standard food medium.

Total numbers emerging were recorded.

Results

The results of the segregation between singed and its wild type allele under low and high density conditions in bottles are illustrated in figure 11. The graph shows the proportion of singed males as a percentage of the total number of males counted each generation. The proportion of singed males in the high density bottle was recorded between generations 4 and 6. The similarity of the decrease in frequency between the two densities from generation 4 to 6 suggests that the singed phenotype was not under more intense selection at high density than at low.

In table 10, the results from the reciprocal crosses, wild females x singed males and singed females x wild males are shown. In all the comparisons the female's genotype was sn/+ and therefore the males in any trial were competing against the same genotype. There were no sex differences within the first two trials over the three densities. Thus singed males did no worse under intense competitive conditions than the wild type males. In the third trial 100 progeny of each reciprocal crosses were put into the same vial and so both types of males were competing against a single female type. Although there was a sex difference in the numbers that emerged, it was an excess of males and there was no difference in the numbers of the two types of males. Since no indication was found to suggest a difference in larval survival between singed and its wild type allele, it was felt that further comparisons of F_2 crosses would not be worthwhile.

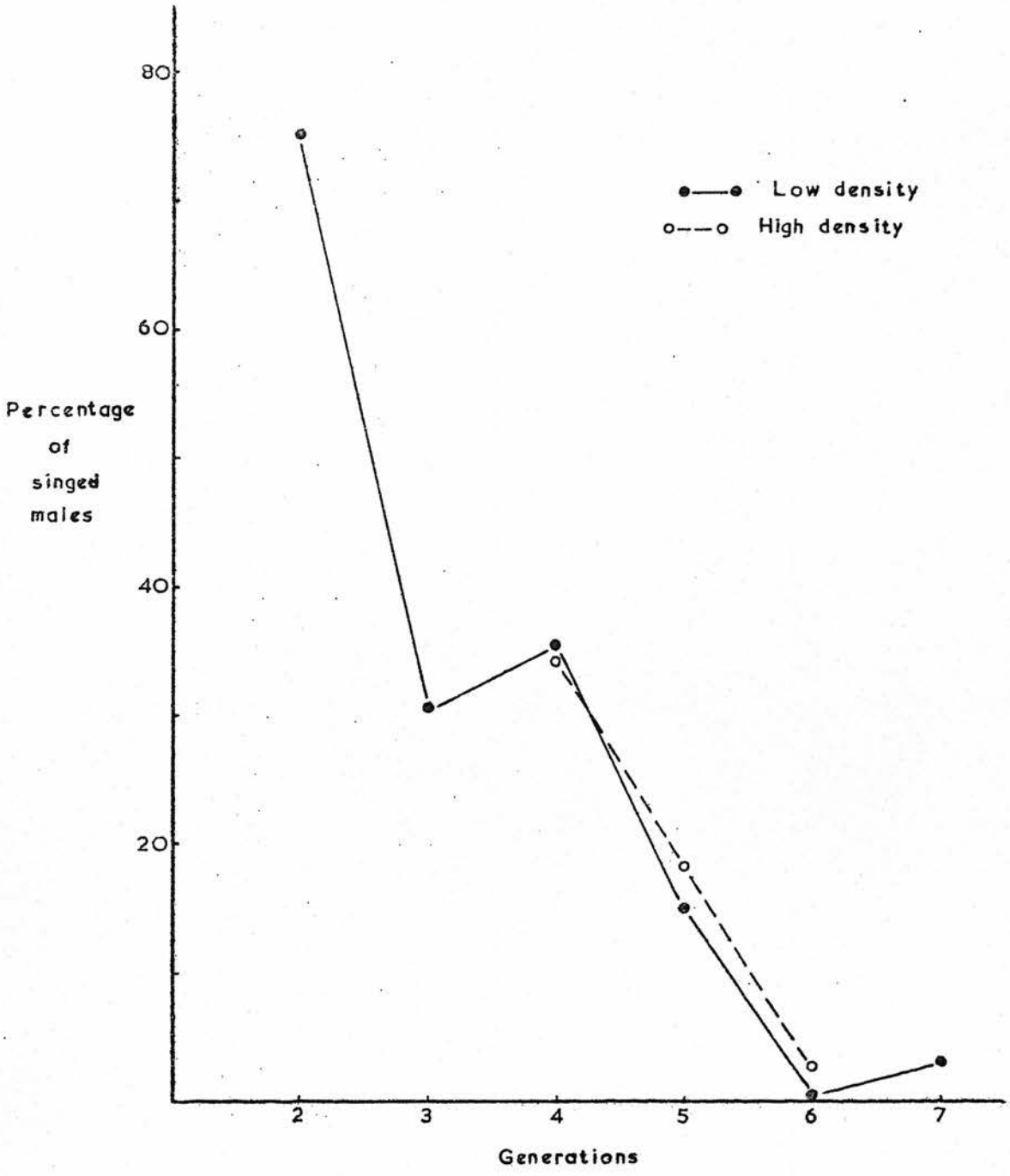


FIGURE II. THE DECLINE IN FREQUENCY OF SINGED MALES WHEN COMPETING AGAINST WILD TYPE MALES AT LOW AND HIGH DENSITY.

Table 10 Competition between singed and its wild type allele

1st Trial	Competitor's Genotype		Numbers Emerging	Chi-square	% Survival
Low Density	sn/+	♀♀	108*	NS	54
(50)	+	♂♂	109		54.5
Intermediate Density (100)	sn/+	♀♀	31o	NS	31.0
	+	♂♂	31		31.0
High Density	sn/+	♀♀	73x	NS	18.25
(200)	+	♂♂	81		20.25
2nd Trial					
Low Density	sn/+	♀♀	137*	NS	68.5
(50)	sn	♂♂	126		63.0
Intermediate Density (100)	sn/+	♀♀	33o	NS	33.0
	sn	♂♂	23		23.0
High Density	sn/+	♀♀	68x	NS	17.0
(200)	sn	♂♂	73		18.25
3rd Trial					
	sn/+	♀♀	14o	9.0	14.0
High Density	sn	♂♂	17	P 0.005	34.0
(200)	+	♂♂	18		36.0

* Total from 8 replicates

o Single vial

x Total from 4 replicates

Discussion

The results from the segregations of scarlet and claret over the six generations in vials are quite clear. The gene frequencies did not decline at a faster rate under highly competitive conditions than under non-competitive conditions. In both cases there is good agreement between the final generation estimates and the value predicted for low density conditions. The results from the segregation between ebony-sooty and its wild type allele are more ambiguous. There is a consistent difference between the low and the high densities which is not found in the case of scarlet or claret. It is possible that a selective effect of density has been found but this is not as large as would have been predicted on the model described.

The results from the claret cages show similar results to those found with the experiment carried out in vials. The survival of the claret genotype is not affected by larval competition when segregating with its wild type allele. The results from the scarlet and ebony-sooty cages are complicated by the evidence of segregation of other background loci. Scarlet has decreased under both cage regimes to below the expected value for extreme larval competition. It is difficult to interpret these results because of the detected background segregation in both the scarlet cages. It might be concluded that these background loci would retard the loss of the mutant through an overdominant effect. However this cannot explain the low frequencies found in the scarlet cages. There will be many other factors such as mating preference, differences in development time and longevity in population cages which could contribute to a rapid decrease in the mutant frequency.

The ebony-sooty cage results show a difference between the levels of larval competition in the two cages indicating that the vial experiment may be a real effect of density. The final frequencies in the cages are lower than expected but as with scarlet there may be other factors contributing to the decrease. This response to different density levels is confirmed by Moree & King (1961) who examined the e^{11} allele of ebony. Dawood & Strickberger (1964) used ebony but they were also interested in the effect of heterozygosity on the relative viabilities. They pointed out that increasing the heterozygosity of loci other than ebony showed no increase in the relative viabilities during competition. These investigations were carried out over a single generation but the results nevertheless have confirmed the outcome when segregation has been followed over many generations in the present investigation.

The egg to adult viabilities in experiment 5 show that the F_1's are superior to both homozygote parents although with ebony-sooty only the females show this superiority. This advantage may well retard the loss of recessive genes and may explain the results of claret and scarlet in the vial experiment but not in the cage populations of scarlet. The ebony-sooty viability is very much reduced although this may have been exaggerated due to experimental reasons. The F_2 segregation data on competition between genotypes give no evidence of density dependent selection in the case of claret and scarlet. There is a deficiency of ebony-sooty genotypes at both densities reflecting a genetic disadvantage. The decrease in the frequency of the singed gene was apparently independent of density levels.

It is claimed that density dependent selection may be an important mechanism in the maintainance of polymorphisms (Clarke, 1972). The problem of conventional genetic load may be solved and thus allow extensive heterozygosity to maintain variation within populations. In practice it may be difficult to distinguish between density dependent selection and selection depending only on phenotype frequency. It has been suggested that the competitive relationships between phenotypes may help maintain variation at enzyme loci (Kojima & Yarbrough, 1967; Kojima & Tobari, 1969). More recently Nassar et al (1973) described an experiment in which frequency dependence was detected only under competitive larval conditions for an inversion polymorphism.

From the mutants examined in this chapter, it is evident that density dependent selection is not a common occurrence at the larval stage in Drosophila. Ebony-sooty would appear to be the only mutant influenced by the density of competing larvae although other contributing factors in population cages cannot be ruled out. Other explanations are required to explain the decrease in the frequency of the mutants - claret, scarlet and singed. Many investigations have concluded that mating preference by females or reduced mating ability by males may be one of the most important factors controlling the frequency of major genes segregating with their wild type alleles (Reed & Reed, 1950; Merrell, 1953; Merrell & Underhill, 1956; Lewontin, 1955; and Bundgaard & Christiansen, 1972). It would seem that this is the most likely explanation for the mutants claret, scarlet and singed.

Conclusions

Although some evidence of density dependent selection was found for ebony-sooty, it is considered not to be of common occurrence. The other three mutants showed no effects of increased larval density. From the evidence of other investigations it is concluded that mating preference is probably the major factor controlling survival of visible mutations competing with their wild alleles.

CHAPTER FOUR

THE EFFECT OF SELECTION ON THE CHARACTER STERNOPLEURAL CHAETA NUMBER

General Introduction

In chapter 2 the environmental effects of larval competition on chaeta number were examined. Indications of stabilizing selection operating on chaeta phenotypes were observed. In chapter 3 some evidence of density dependent selection was found for one of the mutants used, although it was not concluded to be of general occurrence among the major gene mutations examined.

In this chapter the effect of larval competition on different chaeta genotypes is examined for evidence of stabilizing selection. Experiments are set up in an attempt to distinguish between two extreme models of stabilizing selection. The five experiments reported in this chapter are described separately with a final section on conclusions.

Experiment 7 The effect of larval competition on sternopleural chaeta genotypes

Introduction

Various approaches have been used to demonstrate that differences in fitness, as measured by larval viability, are found for different chaeta number phenotypes. Kearsey & Barnes (1970) crossed two lines selected for high and low chaeta number and found that there was an elimination of extreme genotypes when the population was maintained under conditions of intense larval competition. McGill & Mather (1972) crossed two inbred lines and competed representatives from the F_2 chaeta range against a test stock. The extreme chaeta phenotypes had lower competitive abilities than the intermediate phenotypes. In this experiment the effect of larval competition in two highly selected lines for chaeta number and crosses between them will be examined.

Materials and Methods

Reciprocal F_1 and F_2 crosses were constructed from the high (C_3A) and low (DF) chaeta lines. Eggs were collected from each parent line and from each of the F_1 and F_2 reciprocal crosses and transferred to 3" x 1" vials containing 5ml of food. Three egg densities were set up at 100, 500 and 1,000 per vial. Ten replicates were set up for the 100 egg density, except for the reciprocal F_1's where 20 were used, four for the 500 and two for the 1,000 egg density.

At a later date, first instar larvae were used as a check on the hatchability of these lines. Larvae from the parent lines and from their reciprocal F_1 crosses were used. In addition two segregating

generations from the original reciprocal F_2 crosses were used at generation 18. Only one density of 200 first instar larvae was set up in 2ml of food in 3" x 1" vials with five replicates for each parent and reciprocal.

In both the egg and larval densities the number of survivors was recorded and a sample of both sexes were scored for chaeta number.

Results

The survival rates for the egg densities are shown in table 11 and illustrated in figure 12. In contrast to experiment 1, in which the wild Kaduna population was used, the survival rates are very low for the parental and F_1 generations in this experiment. As the survival rate for the C_3A is similar for all densities, it is possible that low hatchability of the eggs has reduced the number of competing individuals. Combining both parents in the F_1 has increased hatchability and produced larger numbers. Both the F_2 generations have survival rates comparable with the Kaduna wild population. If low hatchability is the contributing factor in the C_3A line and assuming less competition among larvae, the mean value of chaeta number should show little change over the densities. The chaeta scores are shown in table 12 and it can be seen that the chaeta score of C_3A has changed by only one chaeta over the three densities. However in the DF line the mean score is highest at the highest density and the F_1 and F_2 generations show a decrease in chaeta score with increasing density.

The variation of chaeta number within each generation has been analysed for homeogeneity using the Bartlett's test. The C_3A is the only line to show homeogeneity of variances over the three densities.

Table 11 Percentage survival of sternopleural chaeta genotypes from egg to adult

	Egg Densities		
	100	500	1,000
C_3A (H)	19.50±2.60	13.05±5.81	12.15±3.15
DF (L)	42.30±3.90	21.80±4.51	12.15±0.65
$F_1(H_{♀} \times L_{♂})$	61.55±4.47	38.95±4.23	24.10±2.60
$F_1(L_{♀} \times H_{♂})$	32.30±3.63	25.15±5.02	26.60±0.90
$F_2(F_1 H_{♀} \times L_{♂})$	84.20±5.27	49.40±5.12	23.55±1.55
$F_2(F_1 L_{♀} \times H_{♂})$	87.22±1.29	59.45±3.95	20.80±1.00

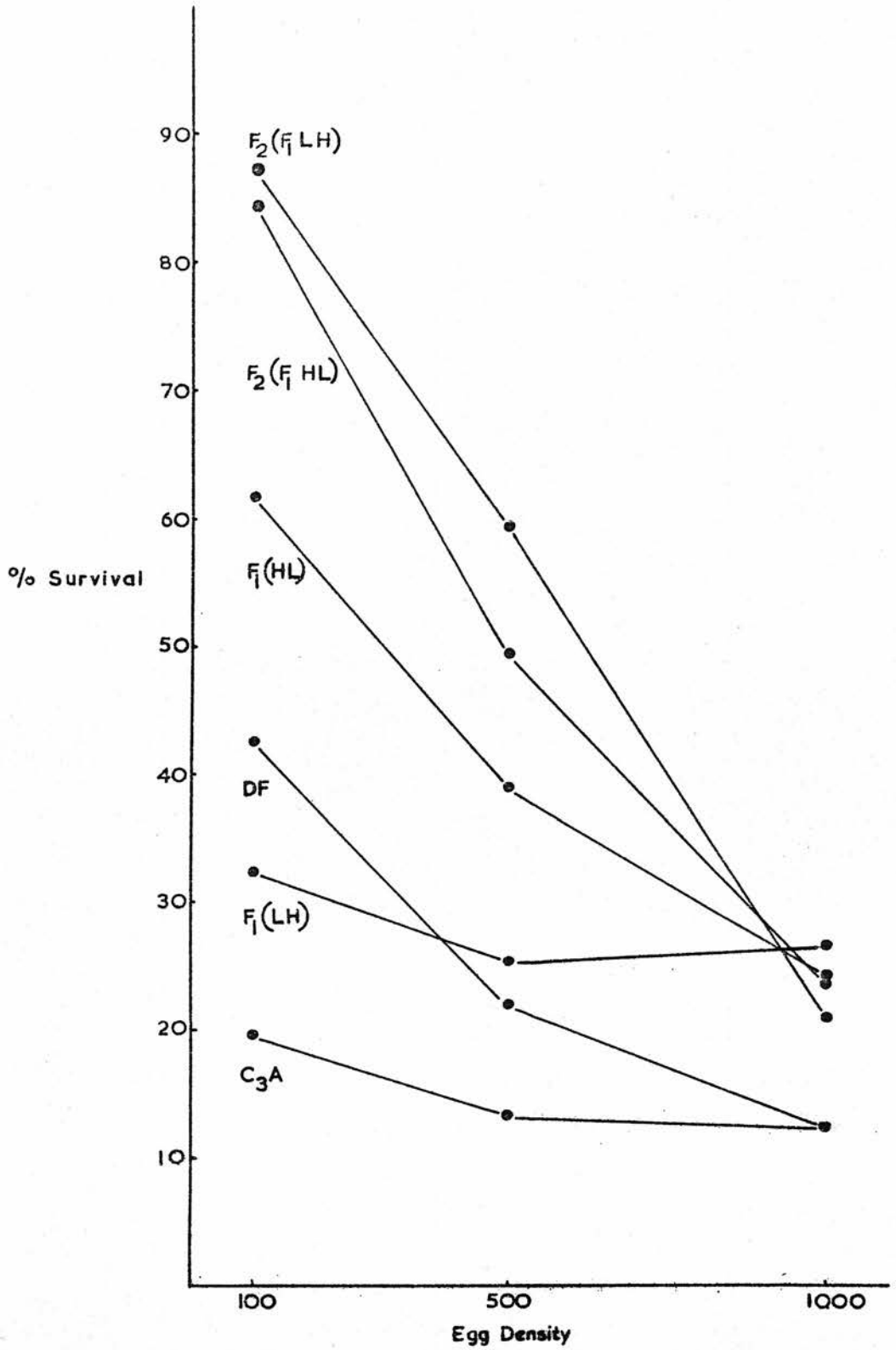


FIGURE 12. PERCENTAGE SURVIVAL OF EGG TO ADULT OF $C_3A(H)$, DF(L) AND THEIR F_1 AND F_2 CROSSES

Table 12 Chaeta scores of survivors (male & female scores combined)

		Egg Density			Bartlett's Test
		100	500	1,000	chi-square
C_3A (H)	$\bar{X}$	49.1105±0.3388	47.7983±0.3088	48.1833±0.3421	
	V_x	21.8131	22.7186	21.0891	0.161 NS
DF (L)	$\bar{X}$	7.700±0.0529	7.8050±0.0500	7.9500±0.0547	
	V_x	1.1478	1.0295	0.6778	18.40***
F_1 (HL)	$\bar{X}$	18.8428±0.1131	17.7725±0.1019	16.2250±0.1228	9.74***
	V_x	4.4938	4.1611	3.0295	
F_1 (LH)	$\bar{X}$	16.0971±0.0969	15.6250±0.0824	15.3025±0.0818	
	V_x	3.2913	2.7512	2.6877	4.60***
F_2 (F_1HL)	$\bar{X}$	16.7810±0.3077	17.1725±0.2817	16.2666±0.2227	62.2***
	V_x	35.0549	31.7671	14.8918	
F_2 (F_1LH)	$\bar{X}$	17.2650±0.2831	15.4975±0.2291	15.0266±0.2002	77.25***
		32.0950	21.0025	12.0594	

*** 1% level of significance

The variation in the DF line and in the reciprocal F_2 generations has been reduced by over a half at the highest density.

Because of the low hatchability in the parental lines it was decided to run a series of competition trials using first instar larvae. The results are shown in table 13. The level used here was equivalent to around 500 eggs in 5ml of standard food medium. It can be seen from table 13 that the survival rate has increased in C_3A by a factor of three. The other generations have also increased in every case indicating the contribution that hatchability made in the first run. The F_1 and F_{18} generations have higher survival rates than the equivalent results at the 500 density in Kaduna. This indicates that hatchability also contributed to lowering the survival rates in the wild population. Although hatchability has considerably reduced the survival rates in the parental generation and to a lesser degree in the crosses, the same ranking of survival rates is found for both egg and larval densities. The one exception to this is the F_1(HL) which had the highest larval survival rate but which was intermediate in survival in the egg density trials.

Discussion

These results indicate that the two selection lines, C_3A and DF differ with respect to the production of fertile eggs and the survival of larvae. Also both lines are much lower in these respects than their crossbred progeny. It is known that sub-vital genes are present in both the parent lines. C_3A has a sub-vital gene on the first chromosome and DF has a sub-vital on the fourth chromosome (Louw, 1966). It would be expected that the parental types segregating out in the F_2

Table 13 Percentage survival of chaeta genotypes from larval densities

	% Survival	Chaeta Score	
		Mean	Variance
C_3A (H)	39.2±7.0	41.48±0.40	15.83
DF (L)	51.9±1.2	8.37±0.10	0.98
F_1 (HL)	79.4±2.8	16.44±0.16	2.45
F_1 (LH)	63.1±7.2	14.35±0.13	1.81
F_{18} (HL)	73.5±3.5	14.94±0.25	6.28
F_{18} (LH)	77.5±2.7	14.56±0.27	7.34

generation would not survive because of low hatchability and reduced larval survival. The distribution of chaeta number of the combined F_2 generations are illustrated in figure 13. Since an F_2 generation was not set up using larvae, the contribution of hatchability cannot be determined with any precision. However 85% of the eggs reached the adult stage at low density and so hatchability must have been very high. At high density it would be expected that few of the extreme individuals would survive when competing with the intermediate types. Since C_3A has a lower survival rate than DF, a greater proportion of high extreme individuals would be eliminated. The distribution at high density in figure 13 would appear to confirm this.

However from the results of experiment 3 it would be predicted that due to an environmental reduction in body size an individual with a score of 46 chaetae would be reduced to a score of 33 at high density. This corresponds almost exactly to the highest individual score at high density. As regards the low scoring individuals, the mean of the DF line when competed on its own in both trials, was highest at the highest density. As this line will be almost completely homozygous this will be an environmental effect. Therefore the environmental effect of competition appears to be restricting the range at both extremes rather than decreasing chaeta number score over the entire range.

It is possible that either an environmental or a selective effect could be the major cause of the restriction in the phenotypic range although both may be acting together. To decide on the role of each of these effects it would be necessary to progeny test the survivors from both densities under the same environmental conditions to see whether the same array of genotypes is present at both densities. This will be carried out in the following experiments.

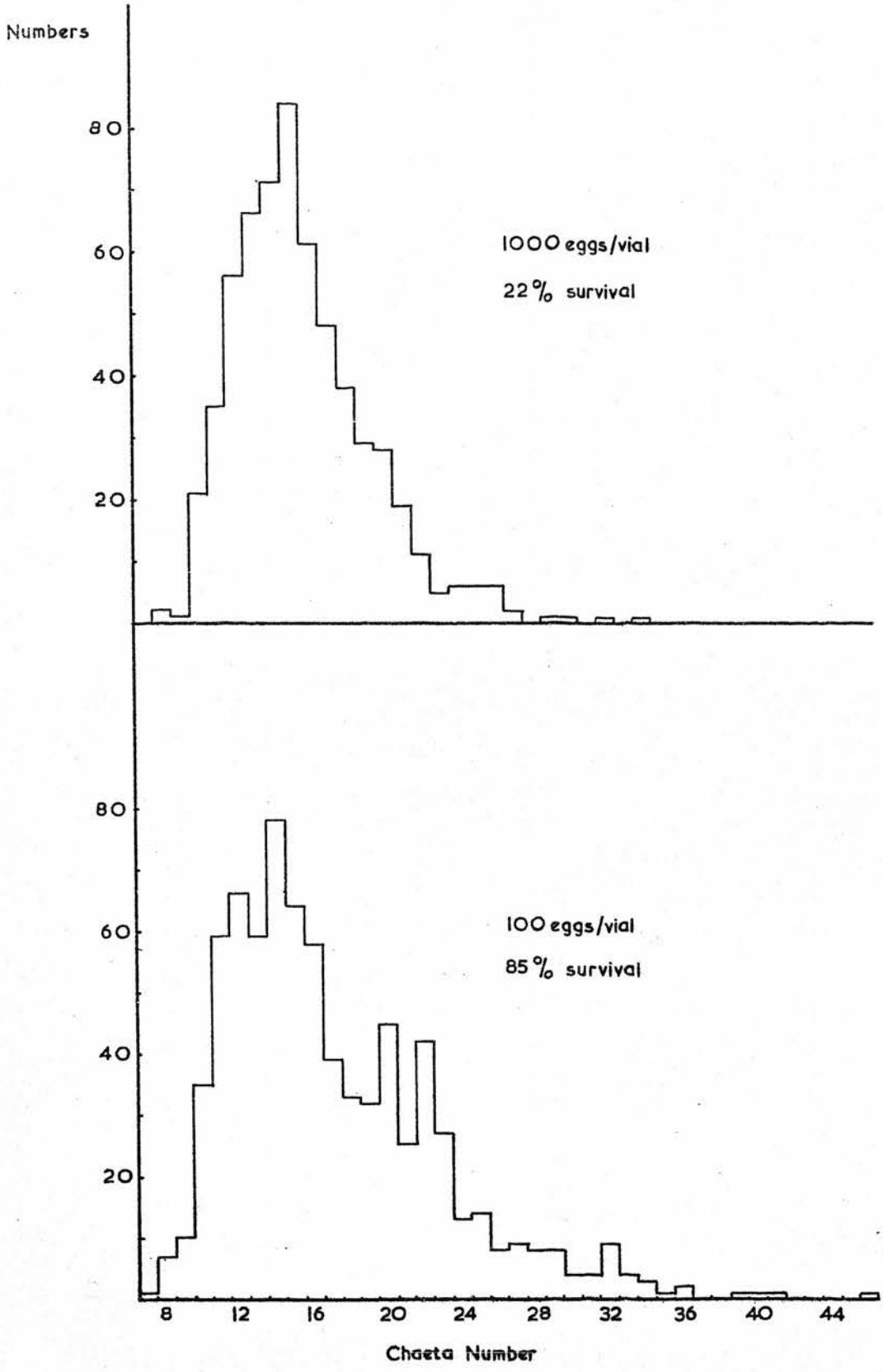


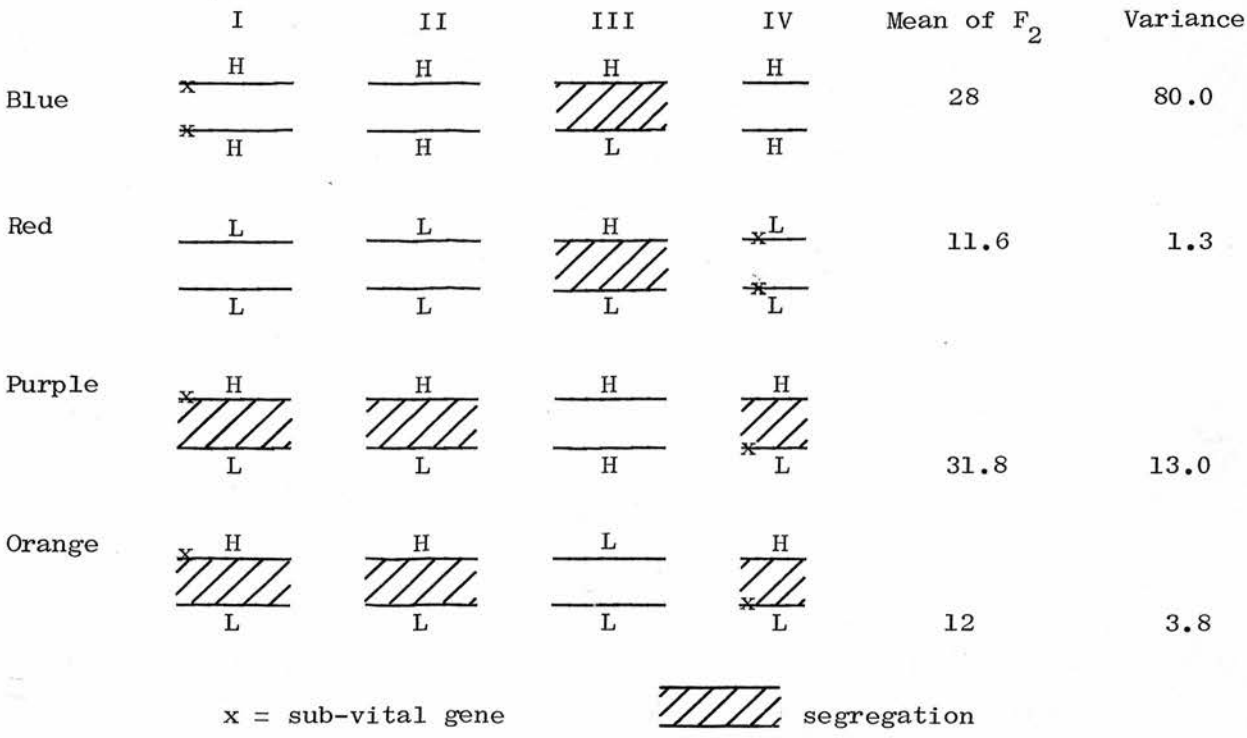
FIGURE 13. FREQUENCY OF CHAETA PHENOTYPES FROM THE COMBINED F_2 GENERATIONS AT LOW AND HIGH DENSITIES.

Experiment 8 Response to larval competition in four synthetic populations

Introduction

In the previous experiment the effect of larval competition on the variance of chaeta number in an F_2 generation was observed. In this experiment the effect of larval competition on both the mean and the variance of chaeta number in four segregating populations will be examined.

Robertson (1967) constructed four populations from the selection lines C_3A and DF which have the following constitution where H = C_3A and L = DF:-



Robertson, argued that if the optimum model of stabilizing selection is correct then the means of the populations should return to the wild type

mean of 18. On the other hand if the homeostatic model is true then there should be no change in the means of the populations. After a period of four years no significant changes had taken place thus indicating that the homeostatic model was a more valid explanation. Selection back to the wild population mean was carried out and a response was obtained in all populations indicating that sufficient variation existed in each population for such a return.

However, in experiment 2 of this investigation it was found that little competition is present during the larval stage in populations maintained on food pots as the above populations had been. If larval competition is associated with sternopleural chaeta number as is claimed (Linney et al, 1971) then it might be expected that the experiment set up by Robertson might not reveal the contribution of larval competition on chaeta number. The purpose of this experiment was to examine the effect of larval competition on the four synthetic populations by maintaining them on a rotational vial system as the results from experiment 2 indicate that intense larval competition is present under such a system.

Materials and Methods

These populations had been maintained in population cages on a system of pots as described in experiment 2, for about four years. A replicate cage was set up for each of the Red, Purple and Orange populations on a system of rotational vials. For convenience, a total number of 24 vials was used instead of 20 as vials could be put into the cage and removed after three weeks in groups of two or four depending on the day of the week. Scores of chaeta number were recorded from

flies sampled directly from the cages. After about a year and a half, samples were scored again from the pot cages and from a low density sample from the vial cages.

As the C_3A and DF lines are fixed for different alleles at ADH, Est-6 and FAP, all the populations were checked for segregation.

The Blue population had become contaminated before starting this experiment and so this had to be reconstructed. Since this population will be examined in more detail than the other populations, the eye colour marker claret was incorporated into the population so that contamination could be easily checked. The parental stocks that were used for the construction of this population were ca-D_3inC which was described in experiment 4 and ca-C_3A. A cross between these stocks produces a population segregating for high and low chaeta number genes on the third chromosome in an otherwise high background. This population will be referred to as ca-Blue. The ca-Blue population was set up in a population cage maintained on the rotational vial system using a total of 24 vials and another cage set up on the 7 vial system. The population was also maintained in three bottle cultures for 24 generations before being maintained as a pot cage. Chaeta number was scored from all three population systems over a period of $2\frac{1}{2}$ years.

Results

The scores from the Purple, Orange and Red populations are shown in table 14. The original F_2 scores are taken from Robertson's data when the populations were set up in 1967. The pot cage scores were recorded in June 1972, some five years after being set up. There has been no significant change in the mean of the Purple and Red cages

Table 14 Chaeta scores in the Purple, Orange and Red Populations

		F_2 score	Pot (June 1972)	Rotational (Dec.1972)	Pot (Dec. 1973)	Rotational* (Dec.1973)	t-values
Purple	$\bar{X}$	31.85	30.92	27.48	31.28	31.77	0.92
	V_x	13.05	9.20	13.04	10.37	15.85	P=20-40%
	N	100	200	200	86	100	
Orange	$\bar{X}$	12.12	14.96	13.79	14.51	14.25	1.29
	V_x	3.80	3.69	2.35	3.24	2.89	P=10-20%
	N	120	200	200	150	150	
Red	$\bar{X}$	11.62	10.89	10.23	10.18	10.25	0.39
	V_x	1.30	1.81	1.77	1.40	1.85	P>50%
	N	100	200	100	100	100	

* Reared at low density

although the mean of the Red cage has decreased slightly. The Orange cage has returned by about 50% to the original wild population mean. After six months (December, 1972), chaeta number was scored on flies sampled directly from the vial cages. In all cases the mean score has decreased in these cages either as a consequence of selection or of environmental depression of chaeta number. To distinguish between these two, individuals must be reared at low density. After 18 months (December, 1973) flies were scored directly from the pot cage and from samples from the rotational vial cages reared at low density. There was no significant difference between the two cage systems. Thus after $1\frac{1}{2}$ years of maintenance under intense larval competition little genetic change had taken place although the mean in the Red cage has remained consistently lower than its F_2 value. Although the mean of the Orange cage had returned by 50% before the start of the experiment, the mean has decreased over a period of the $1\frac{1}{2}$ years by $\frac{1}{2}$ a chaeta in the vial cage.

The results from the ca-Blue cages are illustrated in figures 14 & 15. In figure 14, the means and variances of chaeta scores sampled directly from the cages are shown, each mean is based on one hundred individuals of each sex except in the case of the pot scores at F_{24} and F_{44} where half that number of individuals were scored. The generation time is taken to be $2\frac{1}{2}$ weeks (Crow & Chung, 1967). The mean of the rotational vial cage has declined by 4.5 chaetae and the variance reduced to one third in one generation. In the 7 vial cage the mean has declined less rapidly and has only reached a similar level after about a year. The variance has declined in a similar way although it has taken longer. The mean of the pot cage has declined slowly

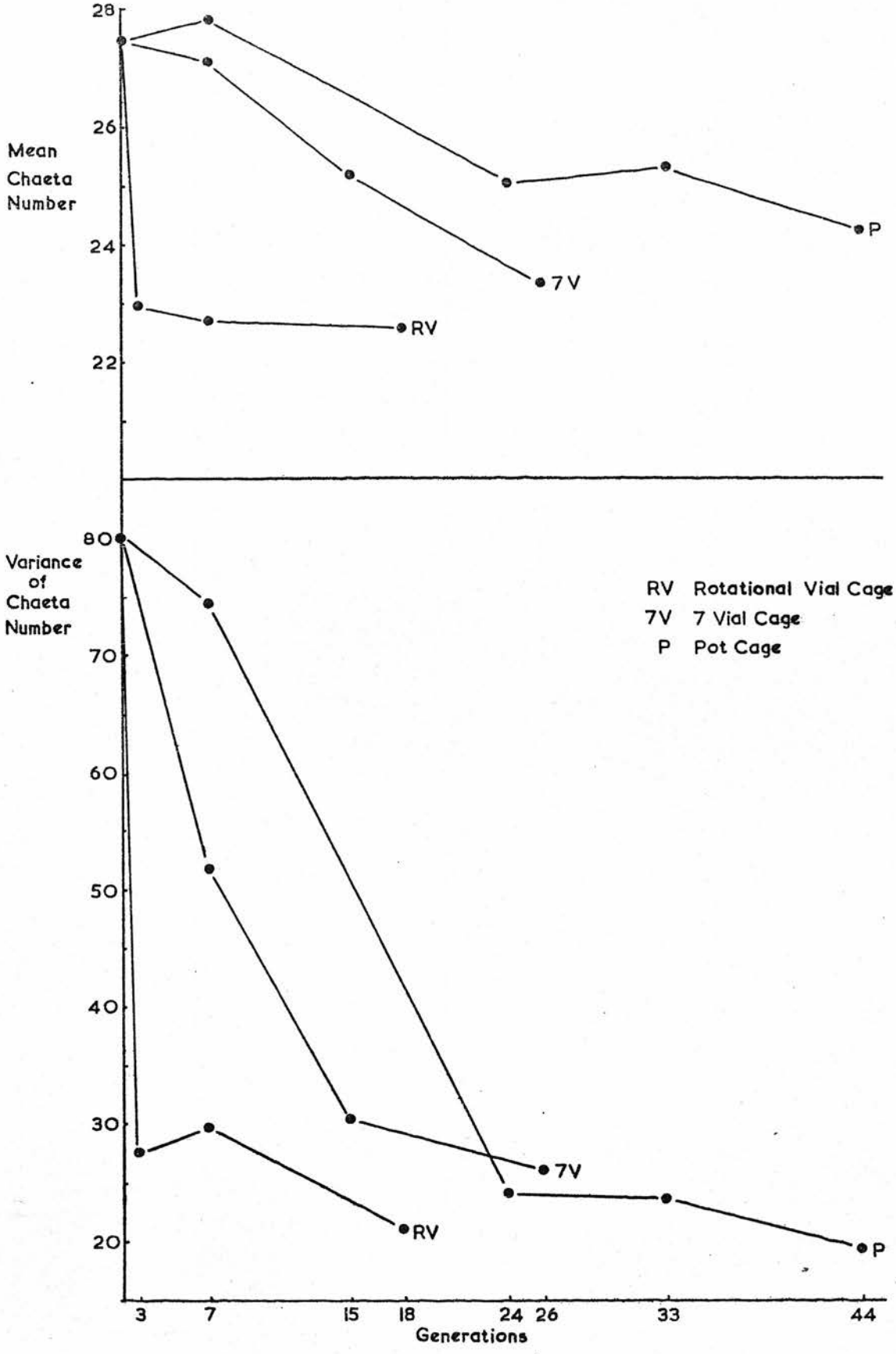


FIGURE 14. THE MEAN AND VARIANCE OF CHAETA NUMBER SCORED DIRECTLY FROM THREE CAGES OF THE ca-BLUE POPULATION.

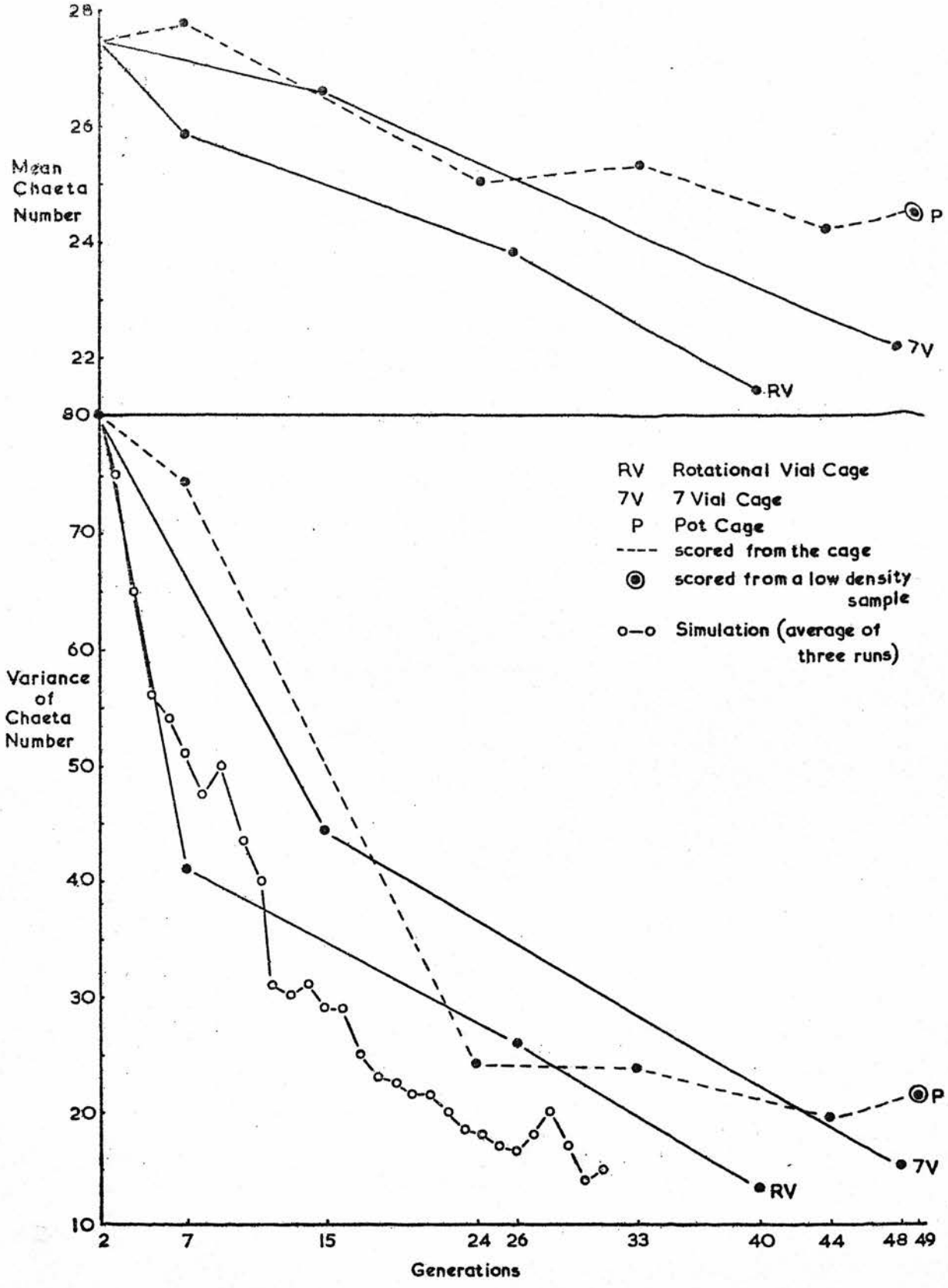


FIGURE 15. THE MEAN AND VARIANCE OF CHAETA NUMBER SCORED FROM LOW DENSITY SAMPLES OF THE ca-BLUE POPULATIONS.

over a two year period amounting to a reduction of about 3 chaetae. The decrease in variance is similar to the 7 vial cage. These changes may be due to both environmental and genetic effects. To distinguish between these effects it was necessary to raise samples from the cages under non-competitive conditions in which the chaeta number character would not be influenced by a reduction in body size. The low density samples are illustrated in figure 15 and in table 15. Any changes here will represent genetic changes. Comparing figures 14 & 15, it is obvious that the steep fall in the mean from the rotational vial cage was purely environmental. However in figure 15 both vial cages have declined in their mean values, the rotational vial cage by 6 chaetae and the 7 vial cage by 5 chaetae. The means scored directly from the pot cage have been shown in this figure as pot cage conditions were found to be equivalent to low density conditions as shown in experiment 2. The last score from the pot cage in figure 15 at F_{49} was measured from a sample reared at low density in a bottle. The difference between the last two means is only 0.2 of a chaeta.

The variances in figure 15 from the three cages have declined in a similar manner, although the decline in the rotational vial cage has occurred at a faster rate. The decline in variance produced from a wide cross for a quantitative character can be simulated by computer. If the number of genes controlling a range of 20 units in the character is set at 8 and the location of the genes on the chromosome and the magnitude of their effects are specified then recombination can be simulated between the chromosome types. The population size was set at 100 gametic types. The simulation was run by Professor A. Robertson and the results are illustrated in figure 15. It can be seen that

Table 15 Chaeta scores in the ca-Blue populations (low density samples)

		Pot (F_{49})	7 vial (F_{48})	t-value (versus Pot)	Rotational (F_{40})	t-value (versus Pot)
Females (100)*	$\bar{X}$	25.46	23.07	3.90	22.02	5.57
	V_x	23.93	13.64		14.24	
Males (100)	$\bar{X}$	23.49	21.26	3.91	20.87	4.86
	V_x	17.08	15.55		12.01	
Total (200)	$\bar{X}$	24.48	22.17		21.45	
	V_x	21.38	15.34		13.39	

* Numbers scored

over a run of 30 generations the time taken to reduce the variation by a half is initially 10 generations and then extends to about 20 generations. The gene pairs furthest apart will attain linkage equilibrium sooner than gene pairs tightly linked. The observed decline in variation has taken longer to reach the expected level. This is probably a consequence of the population size as the effective size of each laboratory population will be around 10-20 times larger than the simulated population.

Discussion

Robertson (1970) concluded from the segregation of these four populations that little genetic change has taken place through natural selection on chaeta number. The results described in this experiment for the Purple and Red populations agree with those conclusions as no change has taken place in these populations for over 5 years. As the Red population carries a sub-vital gene on chromosome 4 the viability is reduced and so the level of larval competition is lowered. In comparison, there is very intense larval competition in the Purple population. A similar situation is found for the Blue and Orange populations. The viability of the Blue population is reduced owing to the sub-vital genes on chromosome 1 whereas Orange was similar in vigour to Purple being heterozygous for these genes.

However the Orange and the Blue populations have shown a change in their means. The mean of the Orange cage had returned by 50% before this experiment was started. There has been a slight decrease in the mean over the subsequent experimental period. The mean would not be expected to return further than 16 as the mean of a population in which

chromosomes 1, 2 and 4 were fixed for C_3A would only be about 15.9. This is the mean of the D_3inC stock. The return in the mean as a percentage of this upper limit is therefore around 60%.

The return in the Blue population maintained on a rotational vial system has also been about 60%. A similar return has been found in the other two Blue populations although the decrease in the mean has been slower.

It might have been expected that as a proportion of males from both the Orange and Purple populations would be less fit since they carry a sub-vital gene on one of their sex chromosomes and therefore chromosomes carrying these genes would be lost together with their complement of high chaeta genes. This has not occurred in either of these populations. Since in the Purple population the mean has remained constant, it is possible that there is an overdominant effect of the sub-vital genes in the females balancing the disadvantage in the male or it may be that the sub-vital genes are not expressed in the males when present on the first chromosome. The increase in the mean in the Orange population is unexplained. However the results from the Orange and the Blue populations do provide evidence for the optimum model which in the case of the Blue population is associated with larval competition.

Since the optimum model of stabilizing selection depends on an interaction between chaeta genes on the fitness scale it has been suggested (Kearsey - personal communication) that the genes in the selected lines C_3A and DF have become coadapted over their past history of selection and inbreeding. Thus the optimum chaeta number may have been altered by selection. If this is true then this would explain the results from the Red and Purple populations.

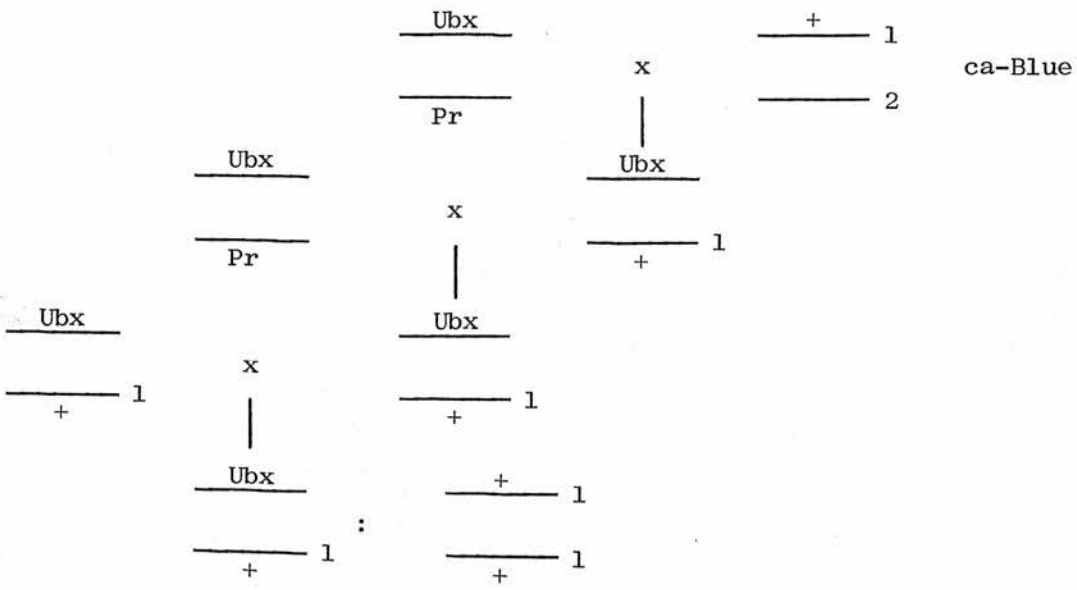
Experiment 9 Chromosome effects in the cage populations of ca-Blue

Introduction

The results from experiment 8 indicate that there has been some genetic change in the three cage regimes of ca-Blue. In this experiment the effect on chaeta number of individual third chromosomes will be measured by extracting these chromosomes from the three cage populations.

Materials and Methods

A stock carrying inversions on the third chromosome was used for extracting chromosomes from the segregating populations. The stock was In(3LR)Ubx^{130}e^{s}/ruPrica and this had been transferred into a C_3A background. The inversion chromosome carries a short inversion on the left arm and a long pericentric inversion marked with Ultrabithorax. The non-inverted terminal region is marked with ebony-sooty (Lewis, 1952). This chromosome is a homozygous lethal and is balanced against the multiply-marked third chromosome ruPrica. The mating scheme was as follows:-



Third chromosomes were extracted from the pot cage at about generation 30 and from the rotational vial cage at generation 26. The 7 vial cage was not sampled. Samples of males from each cage were reared at low density and scored for chaeta number. Representatives from the range 15-36 were mated individually to females of the inversion stock Ubx. The reason for choosing representatives from all chaeta classes was to ensure that a large number of different chromosomal types would be obtained as a wide range was required for the next experiment. This method of extraction will not provide information on the frequency of the different chromosomal types found in the segregating populations but will indicate the types of chromosomes segregating in the populations. Approximately 25 females were scored per line from the pot cage and 5 of each sex were scored for the lines from the vial cage.

In a second extraction of third chromosomes from all three cages at a later date, a random sample of 50 virgin females, which had been reared at low density, were mated to Ubx/Pr males. All three cages of ca-Blue were sampled - the pot cage at generation 44, the 7 vial cage at generation 43 and the rotational vial cage at generation 36. Five individuals of each sex were scored per line. The gene frequency of Est-6 slow and abdominal spot was also recorded from both extractions as an indication of parentage of the lines. The C_3A third chromosome carries the fast allele of Est-6 and the light (lt) allele of FAP. The DF third chromosome carries the other alternatives.

Results

The distribution of the third chromosome chaeta scores are illustrated in figure 16. The top two distributions show the range of

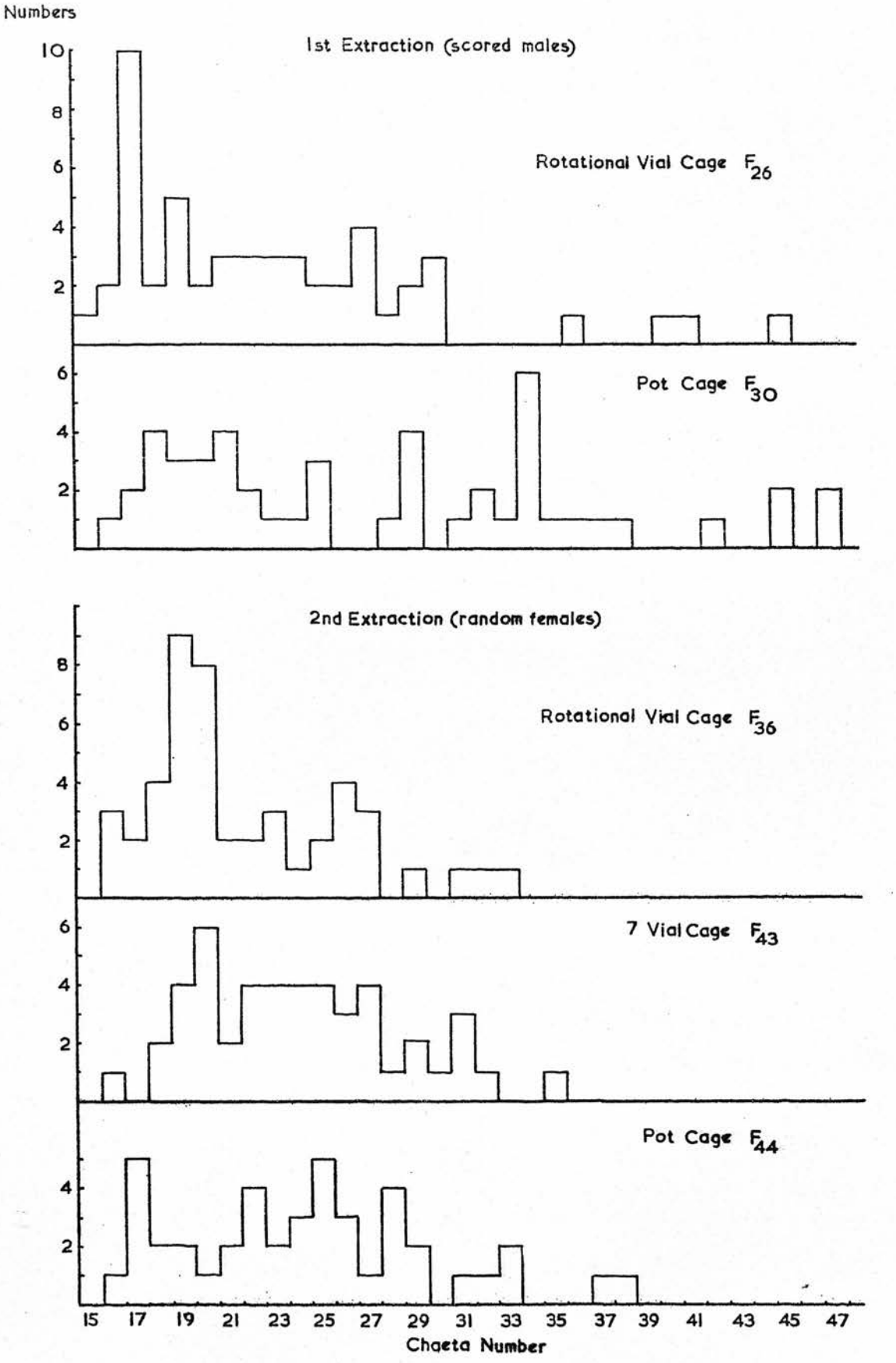


FIGURE 16. THE DISTRIBUTIONS OF THIRD CHROMOSOME CHAETA SCORES EXTRACTED FROM THE ca-BLUE POPULATIONS.

chromosome effects from the first extraction in the rotational vial and in the pot cages. It can be seen that after approximately thirty generations in the pot cage there is still a wide range of chromosome scores. It is likely that both parental chromosomes from DF and C_3A are still present in this population. The lines from the pot cage will be dealt with in more detail in the next experiment. The chromosomes extracted from the rotational vial cage have a similar range suggesting that after approximately 26 generations of extreme larval competition, chromosomes of large effects are still present within the population.

The lower three distributions in figure 16, are from the second extraction and indicate the frequency of chromosomal effects in the three populations of ca-Blue. It is immediately apparent that the frequency of the low scoring chromosomes has increased in the rotational vial cage; more than half the chromosomes from this sample have scores of 20 chaetae or less. This cage has the highest level of larval competition. The highest scoring chromosomes were extracted from the pot cage, the highest chromosome scoring 38. All the chromosomes extracted from the three populations fall within the range of scores from samples of individual flies from their respective cages. This indicates that high scoring chromosomes must be rare or have been eliminated through selection or broken up by recombination. Unfortunately the original females from which the chromosomes were extracted were not scored for chaeta number. If this had been done an indication of the other third chromosome score could have been estimated for each female.

The mean and variances for the lower three distributions in

figure 16 are given in table 16. It can be seen that the mean chromosome effect in the pot and 7 vial cages are similar whereas the rotational vial cage has a significantly lower mean effect. The chaeta number scores on flies sampled from the cages are also shown in table 16. There is good agreement in all the cages except in the 7 vial cage where the mean chromosome effect is much higher than the segregating population score. This could be explained by either a change in the chromosome effect in the five generations between extracting the chromosomes and scoring individuals from the population, or it could be that chromosomes of large effect are still segregating within this population.

The gene frequency of abdominal spot and Est-6 slow are shown in table 16. Both alleles found in the low parent, spt and Est-6 slow, have increased in frequency. It might be expected that these alleles would have reached the highest frequency in the rotational vial cage because this has the highest frequency of low chromosomes but this is not the case. The highest frequency of spt is found in the 7 vial cage. The frequency of Est-6 slow is about the same for the rotational vial and pot cages. The frequency of Est-6 was not measured for the 7 vial cage.

Discussion

If the optimum model is correct then it would be expected that the low DF chromosome would increase in frequency resulting in a decrease in chaeta score. This appears to be the case from the extracted third chromosomes from the rotational vial cage. More than half the chromosomes score 20 or less. This result would also indicate that the low

Table 16 Chaeta scores on third chromosomes extracted from random females from ca-Blue populations

Cage	N	Generation	Chaeta score $\bar{X}$	Chaeta score V_x	Frequency spt	Frequency Est-6 slow
Rotational	47	36	22.21	18.19	0.77	0.76
		(40)*	(21.45)	(13.39)		
7 Vial	47	43	24.46	17.73	0.89	-
		(48)	(22.17)	(15.34)		
Pot	43	44	24.87	29.37	0.86	0.79
		(49)	(24.48)	(21.38)		

* Figures in brackets indicate information from cage individuals given previously in table 15

t-tests	Cage comparisons	t-value	df	P
	Rotational vial versus Pot	2.57	88	0.025-0.010
	Rotational vial versus 7 vial	2.57	92	0.025-0.010
	Pot versus 7 vial	0.40	88	>0.50

chromosomes are increasing in frequency at a faster rate under intense larval competition than under low density conditions.

It is possible that either selection is operating on chaeta number genes through differences in larval survival or that extreme individuals for chaeta number, being more homozygous, are being selected against. If the latter is the case then there would be no change in the population mean as the F_1 types would have the highest fitnesses. However it may be that the parental lines differ in their ability to survive competition. The survival rates of the parental lines of ca-Blue can be compared. The low parent, ca-D_3inC, which was used in experiment 5, figure 10 has a survival rate of 34% at a density of 100 eggs in 1ml of food. The egg to adult survival in the high parent, C_3A, was 13% at a density of 500 eggs in 5ml of food. These survival rates indicate that the C_3A has a very much reduced hatchability in comparison to ca-D_3inC. No larval densities were set up using ca-D_3inC and therefore no comparison can be made with C_3A on larval survival rates. The difference in hatchability between the parent types would explain the decrease in the proportion of C_3A chromosomes in the pot cage. In the rotational and 7 vial cages, differences in larval survival between the third chromosomes might also be present. Since the mean score of the chromosomes from the rotational vial were very low it must be assumed that sub-vital genes affecting hatchability and larval survival are tightly linked to genes increasing chaeta number. As recombination continues, chromosome segments containing sub-vital genes plus increasing chaeta genes will be replaced by alternate segments from the low chromosome.

On the other hand the results obtained in this experiment would substantiate the explanation put forward in the optimum model of stabilizing selection. The mean of all three ca-Blue populations have changed in the direction expected on this model and further, the largest return to the optimum mean value was observed in the cage with the highest level of larval competition. The relationship between larval competition and third chromosome chaeta effect will be examined in the next chapter.

Experiment 10 Examination of the optimum model of selection

Introduction

The results from experiment 7 indicated that flies which were extreme for chaeta number were less fit than their crossbred progeny when their survival was compared at different densities. In experiment 8, it was found that there had been a genetic change in the ca-Blue populations which were segregating for low and high chaeta number under different levels of larval competition. Also in experiment 9 it was found that the low scoring chromosomes had increased in frequency. This increase was highest in the cage with the highest level of larval competition. The results from these experiments suggest that there may be a relationship between chaeta number and fitness which is the result of larval competition.

It is essential to find out how this apparent differential survival of chaeta phenotypes comes about. Either extreme phenotypes have a lower survival rate because they possess extreme values of chaeta number *per se* or because they are more homozygous than the intermediate phenotypes. Robertson (1956) suggested that "if the first model is correct, then on inbreeding to complete homozygosis the more extreme lines should be less fit than the intermediate ones". Linney *et al* (1971) carried out an experiment along these lines in which they chose four inbred lines differing in chaeta number and examined their survival during larval competition. They found that the extreme chaeta lines did not survive when in competition with intermediate scoring lines.

From the distribution of third chromosomes extracted from the

rotational vial cage there is an indication that chromosomes of different effects have different survival rates.

The following two experiments will examine any differences between representatives of third chromosomes from the ca-Blue population.

a) Differences in larval survival during competition

Materials and Methods

The lines used in this experiment were chosen from the third chromosomes lines extracted from the ca-Blue pot cage at generation 30 in the previous experiment. As the first, second and fourth chromosomes are homozygous for C_3A, the third chromosome extractions provide homozygous lines differing only in the proportion of increasing or decreasing chaeta number genes. Twenty of these lines were chosen in such a way as to give a similar mean and variance to the original segregating population.

The design of the experiment, to test for differences between the twenty lines, was as follows. Eggs were collected from inseminated females from each line and were incubated for 24 hours. First instar larvae were collected from each line and set up at low or high density in a random sequence in 3" x 1" glass vials containing 5ml of standard food medium. Low density conditions were set up with 5 larvae from each line giving a total of 100 larvae per vial using thirty replicates. The high density conditions were set up with 25 larvae from each line giving a total of 500 larvae per vial using six replicates. The numbers of survivors of each sex were counted as they emerged. Survivors were collected from the high density vials over a period of ten days. Two hundred adults of each sex from each density were

scored for chaeta number. Also one hundred males from each density were weighed.

It has been established from chapter two that one of the consequences of larval competition is a reduction in chaeta number. Since it is a genetic change which is of importance in this experiment all the survivors from low and high densities must be compared under non-competitive conditions to assess their true breeding value for chaeta number. Representatives from both densities were chosen over the range of survivors and crossed to a tester line. Individuals of each sex were tested and one replicate was set up for each chaeta number. Only representatives of odd numbers of chaeta were tested over the range 15-39. The tester line used was the D_3inC stock which carries the low DF third chromosome in a high, C_3A background. The mean of this tester stock is 15.92 ± 0.22. Four offspring of each sex were scored for chaeta number from each vial at both densities.

Results

Chaeta scores for the twenty homozygous lines are shown in table 17 together with the results of segregation at Est-6 and FAP. It can be seen from the table that the extreme chaeta scoring lines are likely to be original parental chromosomes. If the optimum model holds it would be expected that lines 3 & 4 would have the highest fitnesses, and fitness or survival would decline in either direction. The highest three lines will have the lowest survival values on this model.

The chaeta scores of the survivors from low and high density conditions are illustrated in figure 17. The mean of the distribution at high density has been reduced by $3\frac{1}{2}$ chaetae and the variance by

Table 17 Twenty homozygous lines extracted from the ca-Blue Pot cage at F_{30}

Line Number	Original ♂ Score	Mean of each Chromosome line	Variance	Est-6 F	Est-6 S	FAP spt	FAP lt
1	26	16.16	1.22		x	x	
2	22	17.16	1.37		x	x	
3	26	17.85	1.98		x	x	
4	16	18.33	3.96		x	x	
5	15	19.64	2.87		x	x	
6	20	21.18	3.15		x	x	
7	27	21.91	2.90		x		x
8	36	24.76	3.77		x	x	
9	18	28.75	6.37	x		x	
10	25	31.64	11.49		x	x	
11	36	32.45	4.83		x	x	
12	36	32.96	4.46		x		x
13	31	34.17	6.70		x		x
14	33	35.46	6.69	x			x
15	36	36.83	6.75	x			x
16	31	38.44	7.00	x			x
17	20	42.04	8.46		x	x	
18	28	45.44	16.26	x			x
19	28	46.90	7.36	x			x
20	33	47.00	13.80	x			x

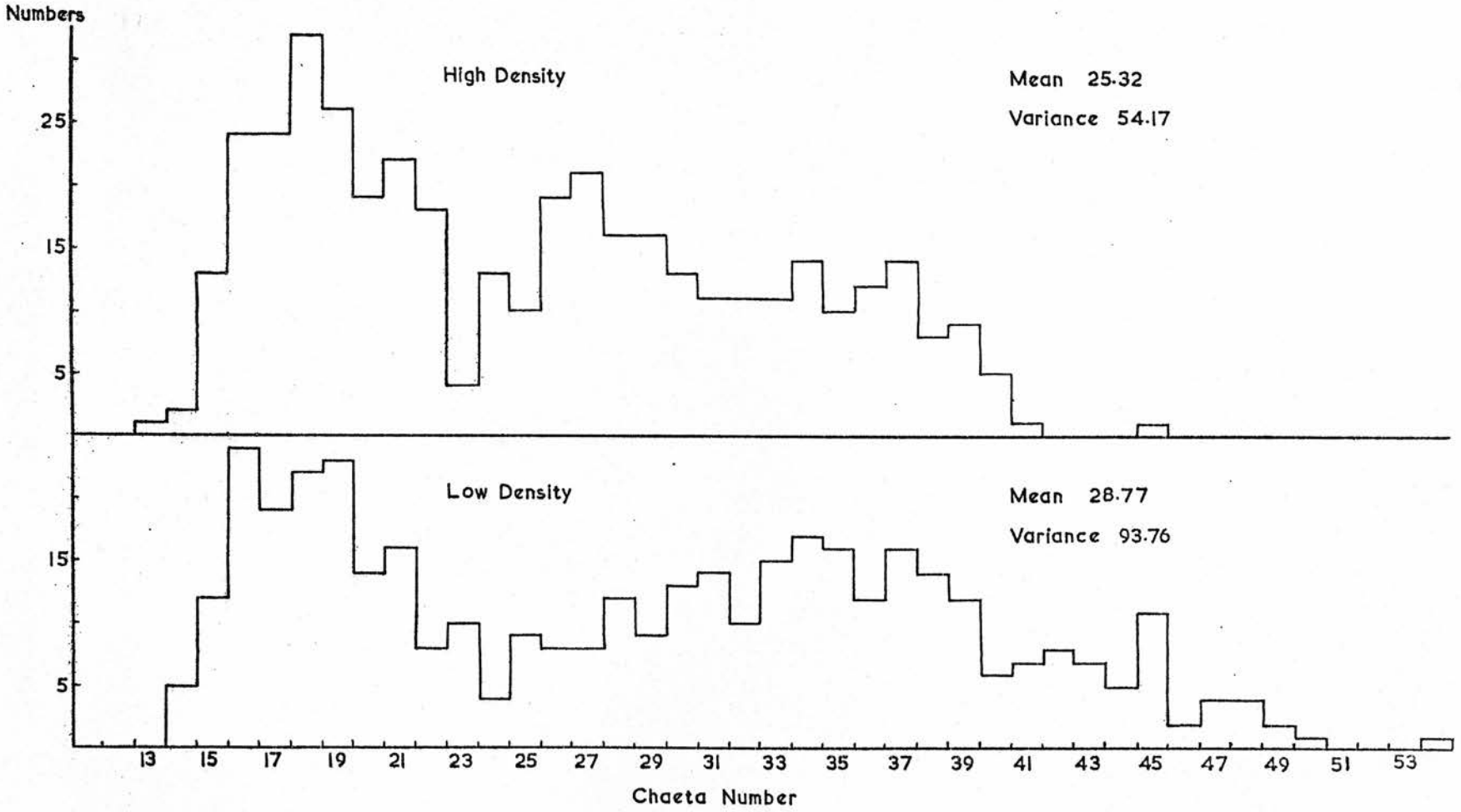


FIGURE 17. THE DISTRIBUTIONS OF CHAETA NUMBER FROM SURVIVORS OF TWENTY HOMOZYGOUS LINES REARED AT LOW AND HIGH LARVAL DENSITIES.

almost a half. The survival rate and average weights of males from the two densities are as follows:-

	Low	High
% survival	85.87	45.40
Average ♂ weight (mg)	0.977	0.606

At the high density the survival rate has been halved and the average body weight has been considerably reduced.

The data from the progeny tests for both densities have been illustrated in figure 18 for females and figure 19 for males. Regression analyses of offspring mean chaeta score on their mother's scores and offspring mean score on their father's score are shown in table 18 together with the regression coefficients for each line.

There can be two explanations for the reduction in the distribution of scores at high density:- either there has been a selective elimination of extreme genotypes, in which case the regression of offspring on parent from high density would be lower or equal to the regression from low density.

Thus:-

$$b_H \leqslant b_L$$

or there has been an environmental restriction of the phenotypic range with no selective elimination of extreme genotypes. The regression slope from high density should be greater than the regression from low density,

Thus:-

$$b_H > b_L$$

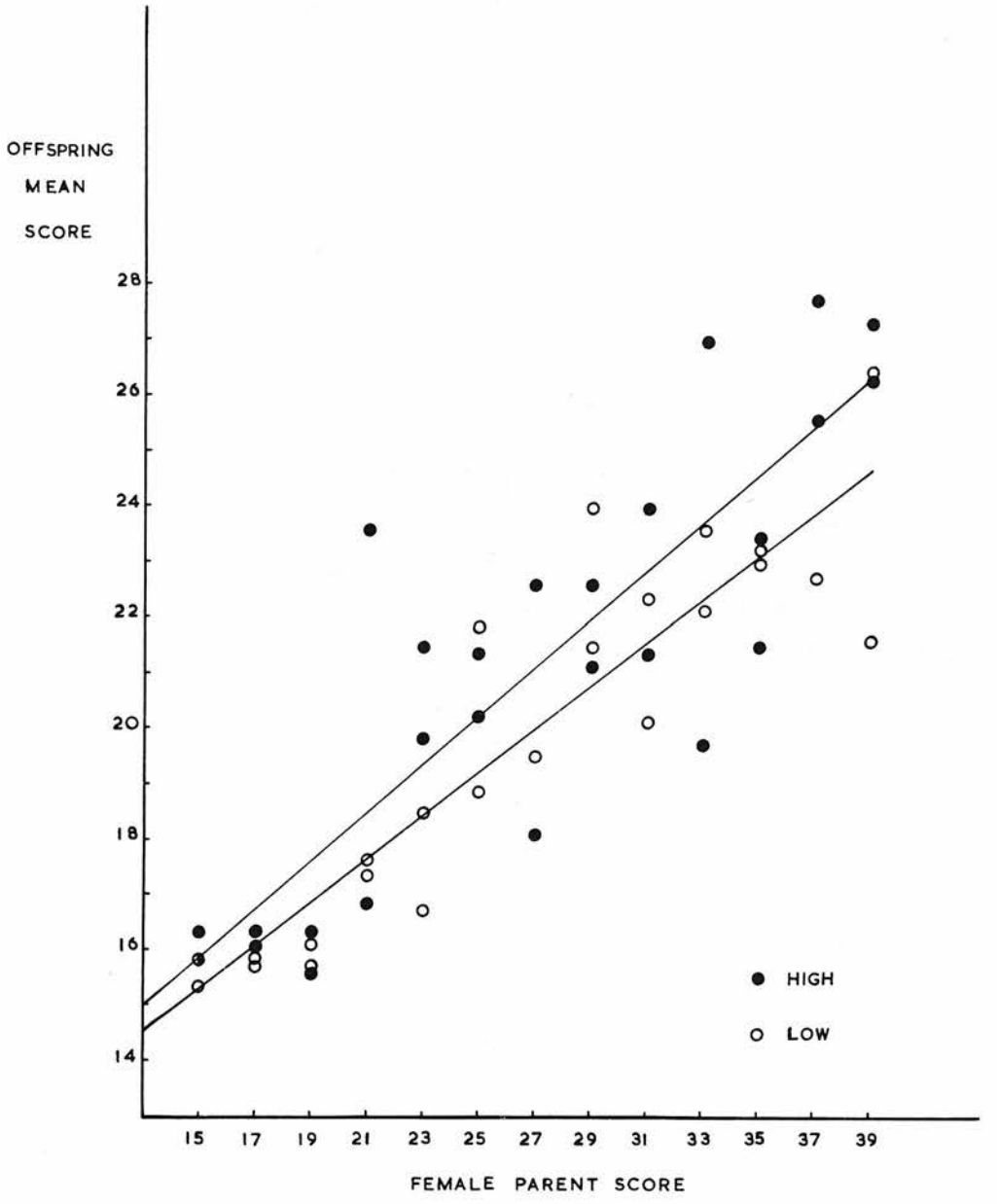


FIGURE 18. PROGENY TEST OF FEMALES FROM LOW AND HIGH DENSITIES:
REGRESSION OF OFFSPRING ON FEMALE PARENT

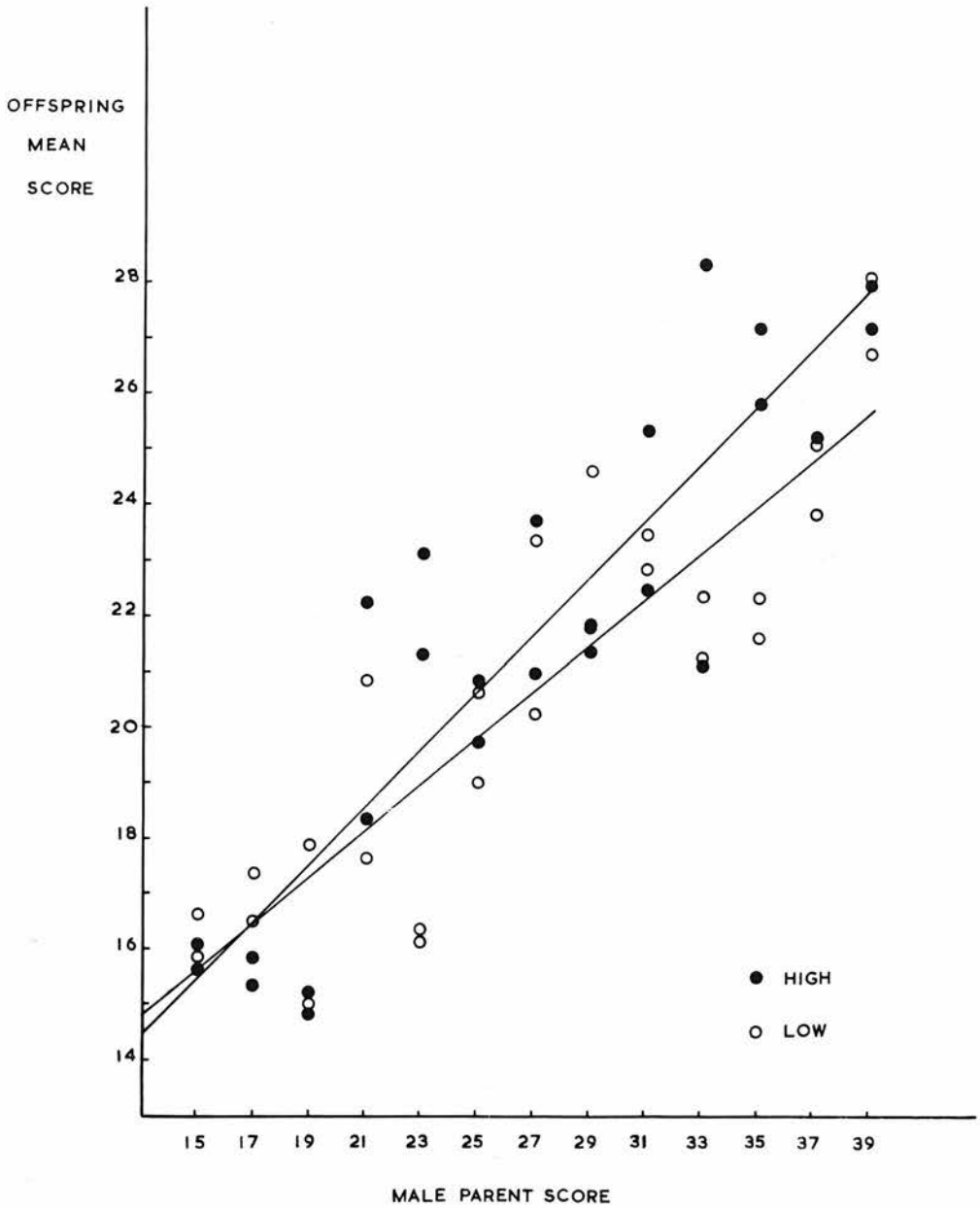


FIGURE 19. PROGENY TEST OF MALES FROM LOW AND HIGH DENSITIES:
REGRESSION OF OFFSPRING ON MALE PARENT

Table 18 Regression analysis of progeny test data

		Females			Males		
	Item	df	MS	P	df	MS	P
Low Density	Regression	1	1707.7266	<0.0001	1	2034.4632	<0.0001
	Remainder	24	12.7958	<0.005	24	23.7848	<0.005
	Within families	182	3.1168		182	4.4384	
	Total	207			207		
High Density	Regression	1	2170.3968	<0.001	1	2827.7312	<0.0001
	Remainder	24	33.6072	<0.005	23	30.9432	<0.005
	Within families	182	3.7376		173	4.7056	
	Total	207			197		

Regression coefficients

	Females	Males
Low	$\hat{b} = 0.3829 \pm 0.0331$	$\hat{b} = 0.4179 \pm 0.0452$
High	$\hat{b} = 0.4316 \pm 0.0537$	$\hat{b} = 0.5147 \pm 0.0538$

t-test $t_{48} = \frac{0.0487}{0.0631} = 0.7720$ $t_{47} = \frac{0.0968}{0.0703} = 1.3776$

P>0.40 P>0.10

From table 18 it can be seen that the regression coefficients are not significantly different in either sex. However the slopes of the regression lines in each sex are numerically greater at high density than at low. From figures 18 & 19 it can be seen that the survivors scoring 35-39 have higher offspring means on average from the high density conditions than from low. This would indicate that the high lines have been environmentally decreased in score and not eliminated.

The distribution of chaeta scores at high density can be corrected for environmental depression and compared to the low density distribution. The correction factor from experiment 3 will be used although the competitive conditions under which this was measured may have been more extreme. It was found in experiment 3 that the survival fell from 75% at low density to 38% at high and the average weight of males at low was 0.86mg and this was reduced to 0.60mg at high density. These figures agree fairly well with the survival rates and average weights of males in this experiment. The corrected distribution is illustrated in figure 20. The mean of this distribution and the mean of the low density distribution differ by only one chaeta and the variances are very similar. It is more likely that there has been a purely environmental reduction in the phenotypic range of chaeta number.

On the other hand, since the regression slopes are not significantly different it might be argued that the top scoring two or three lines have been eliminated. The fact still remains that out of the twenty lines subjected to larval competition representatives of 17 of them have survived. Individuals with 40 chaeta have survived competition with supposedly optimal scores of around 18 chaetae.

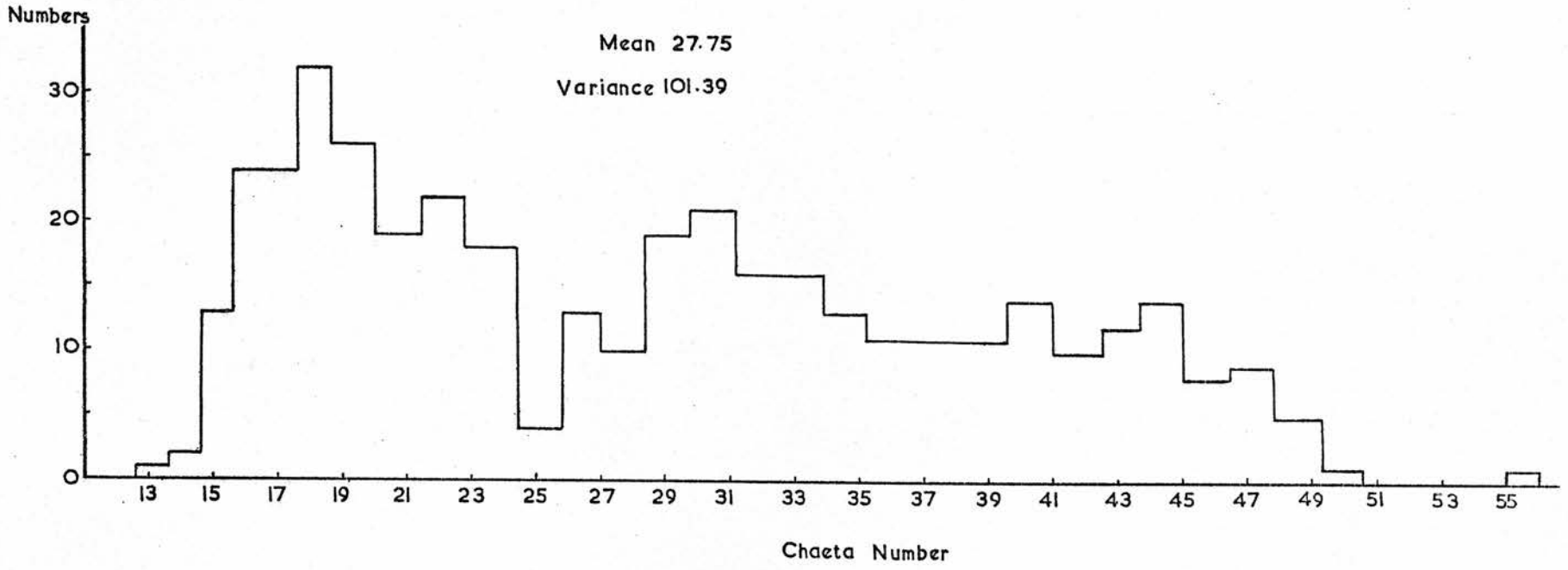


FIGURE 20. THE DISTRIBUTION OF CHAETA NUMBERS OF SURVIVORS FROM HIGH DENSITY CORRECTED FOR ENVIRONMENTAL DEPRESSION.

It is probable that the top scoring lines will be representatives of the C_3A line and therefore may be less fit as was found in experiment 7. This decrease in fitness may not be associated with chaeta genes but may be due to an accumulation of sub-vital genes during the formation of this line.

Discussion

There is little indication from the results that the majority of the homozygous lines used differ in larval survival when in competition with each other. Since the two regression lines do not differ significantly it could be argued that the highest scoring two or three lines have been eliminated. These lines are likely to be representatives of the C_3A parental line. From table 17 it can be seen that the lines 18, 19 & 20 have chaeta scores 45.4, 46.9 & 47.0 and each line carries the fast allele for Est-6 and the 1t allele of FAP.

It might be argued that the original C_3A third chromosome has the highest probability of containing some genes reducing viability which have been lost through recombination in the other homozygous lines.

There are difficulties inherent in the design of this experiment. In the optimum model the chaeta number which is attributed with the highest fitness in this experiment is not in the centre of the range but close to the lower end. The regression line at high density will be fixed at its lower end and the line will rotate around this fixed point. It may then be more difficult to detect differences between selective elimination and environmental effects in the high lines.

Secondly the regression line from the high density progeny tests is known with less precision in comparison to the low density.

Individuals chosen from high density conditions will show a greater variation in body size as would be predicted from experiment 2. Thus although individuals are phenotypically similar in regard to chaeta number their breeding values may be different. The variance between families with the same parental scores will be inflated at high density. In table 18 it can be seen that the remainder mean square for females at high density is almost three times that at low and for males the difference is about one and a half times. The remainder mean squares for all the regressions are significant reflecting the increased environmental variance in chaeta number due to the lines being almost completely inbred.

By using the low DF third chromosome in the D_3inC stock to progeny test the survivors from the two environments the scale over which the regression slopes were compared has been restricted. This is a consequence of the scale of measurement. There is dominance in the direction of low chaeta number on this arithmetic scale and therefore this will further decrease the detection of a difference between the regression slopes. The reason for using the D_3inC stock was that crossbred progeny would have low chaeta numbers and therefore larger numbers of individuals could be scored. However this strategy has not been successful and in retrospect it would have been better to have used a tester stock with a higher mean such as K_{13}inC and score fewer progeny.

An improved experimental design would be that used in experiment 3 where only extreme chaeta scores were used and survivors were progeny tested using the K_{13}inC stock. It is hoped that at a later date this experiment will be carried out.

Experiment 10 b) Differences in egg fertility and oviposition rate

Introduction

Since larval survival does not differ among the majority of the lines in the last experiment it may be that a difference in the fertility of eggs or oviposition rate may influence the survival of the different lines as suggested from the results in experiments 7 & 9. This experiment is designed to test for these possible differences.

Materials and Methods

The experiment was carried out as before at a later date using only 16 of the lines. Lines 4, 11, 16 and 20 were not included because of contamination or loss. Populations of inseminated females from each of the lines were placed into two cages, which varied in the amount of food provided. The low density cage had 20 vials containing 5ml of standard food medium. Into this cage was placed 25 inseminated females from each line, giving a total female population of 400. The high density cage had 6 food vials and contained the same number of females. The females in the low cage were allowed to lay eggs for three days and were then discarded. The females in the high cage were allowed to lay eggs for 8 days and then discarded. 225 individuals of each sex were scored from both cages. Males over the range 15-39 were progeny tested by using the tester stock, D_3inC. Samples of males were weighed from each density. The purpose of this experiment was to test whether there was any difference due to female fertility, oviposition rate or hatchability between the lines.

Results

The results from the second experiment carried out in cages are

illustrated in figure 21. There has been some reduction in variance but the mean has been reduced by only one chaeta. The weights of males from the low and high cages are 0.71mg and 0.61mg respectively. These weights would suggest that body size has been reduced at both densities. The low density weight for males in the previous experiment was 0.977mg. Thus body size has been reduced by almost one third in the low density cage.

This time only males were progeny tested using the D_3inC stock. The regression coefficients from the cages are as follows:-

Low	High
$\hat{b} = 0.4271 \pm 0.0425$	$\hat{b} = 0.4270 \pm 0.0371$

Since the regression slopes are the same it might be that some selection of extreme genotypes has taken place. However, since competition has also taken place at low density it is unlikely that there has been any change in the array of genotypes at either density.

Discussion

The reason for competition to have occurred in the low density cage is to be found in the egg laying behaviour of the flies. In the low density cage it was found that flies tended to congregate into a few vials and lay a large number of eggs while leaving other vials with only a few eggs laid in them. This was also observed in the high density cage, where there were large differences between the number of eggs laid in different vials. It is likely that larval competition occurred under both cage systems resulting in similar distributions of survivors. From figure 21 it can be seen that most of the lines which

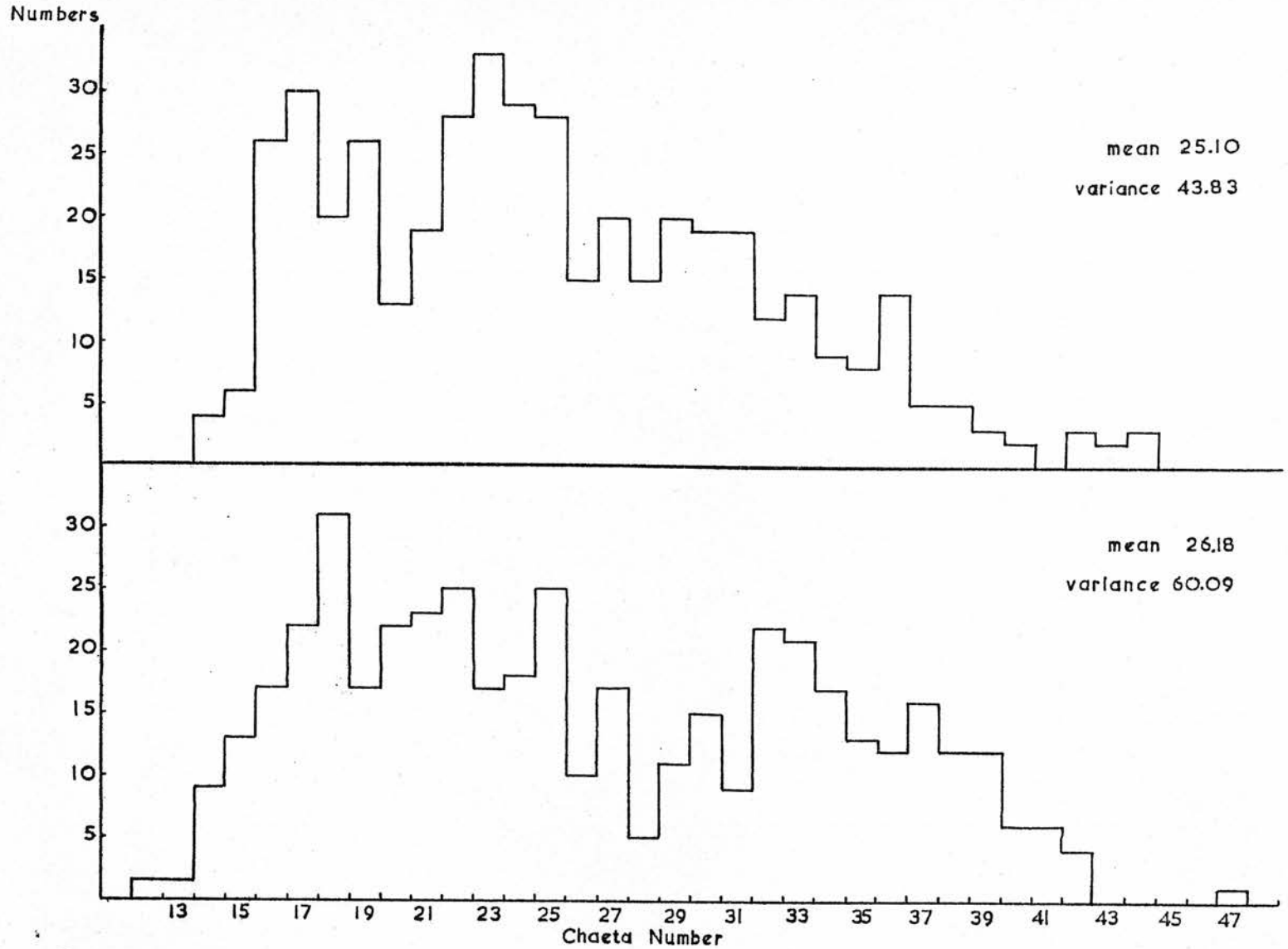


FIGURE 21. THE DISTRIBUTION OF CHAETA NUMBER FROM SURVIVORS OF 16 HOMOZYGOUS LINES REARED UNDER DIFFERENT CAGE CONDITIONS.

occur in the low density distribution also occur at high. However if the low density distribution is compared to that of the twenty lines at low density in figure 17 in the previous experiment, it can be seen that few of the extreme high lines are represented in figure 21. This could be explained by the evidence of competition at low density in the cage population but when the regression coefficients of males from both experiments are compared they are found to be the same. This indicates that there has been an elimination of some extreme genotypes in the cage populations. It can be concluded from this that hatchability is a contributing factor to the survival of the extreme high lines which will be representatives of the C_3A selection line.

Experiment 11 Differences in competitive ability of twenty homozygous lines

Introduction

In the preceding experiments all the homozygous lines were put into competition together. No examination was made of individual fitnesses of these lines. In this experiment each line will be assessed for fitness by competing it against a common tester stock.

Materials and Methods

In this experiment the twenty lines in table 17 were crossed to the inversion stock Ubx^{130}/ruPrica. Males and females carrying the Ubx^{130} inversion balanced against a single ca-Blue chromosome were set up separately in bottles. Each line was run against Ubx^{130} for five generations. In the first, third and fifth generations the percentage of claret was counted. By the fifth generation some crossing over had taken place at the right end of chromosome three and non-parental types Ubx-ca and wild type were found. In this generation the claret marker was ignored and individuals were scored as either Ubx or wild type.

The two original parental stocks which had been used to set up the ca-Blue population were also allowed to compete with Ubx. The two stocks were ca-D_3inC which was the low parent and ca-C_3A, the high parent. The proportion of these types was recorded for the first and last generations. The parents were allowed to lay eggs over three days using all the progeny as parents from the previous generation. Thus there will be some larval competition but this will not be as extreme as vial cage conditions.

Results

The percentages of claret in each line for generations 1, 3, & 5 are shown in figure 22. The expected percentage of claret will be 33% as Ubx is a homozygous lethal. The lines have been ranked in order of chaeta number. It can be seen that there is no difference between the lines as regards their competitive abilities as related to their chaeta scores. Lines 6, 13, 18 and 20 with chaeta scores 21.18, 34.17, 45.44 and 47.0 respectively have the lowest percentage survivals. On the other hand lines 3, 4, 16, and 19 with chaeta scores of 17.16, 17.85, 24.76, 38.44 and 46.90 respectively have the highest percentage survivals. The highest survival of all is line 16 which has a chaeta score of 38.44. The highest chaeta line has the lowest survival rate and, in fact, this stock was eventually lost whereas the next highest line which has about the same chaeta score has an above average survival.

Discussion

These results support the findings of experiment 10a. No consistent difference associated with chaeta number was found. The line which had the highest competitive ability was a line possessing a high chaeta score some 20 chaetae above the wild type value. The difference between the two highest scoring lines must be due to some other effect apart from the chaeta genes themselves.

An unexpected result is that of ca-C_3A, the high parent. The final frequency of the C_3A chromosome is much higher than would have been expected from previous results in experiment 10b. Since some crossing-over took place at this generation these results should be

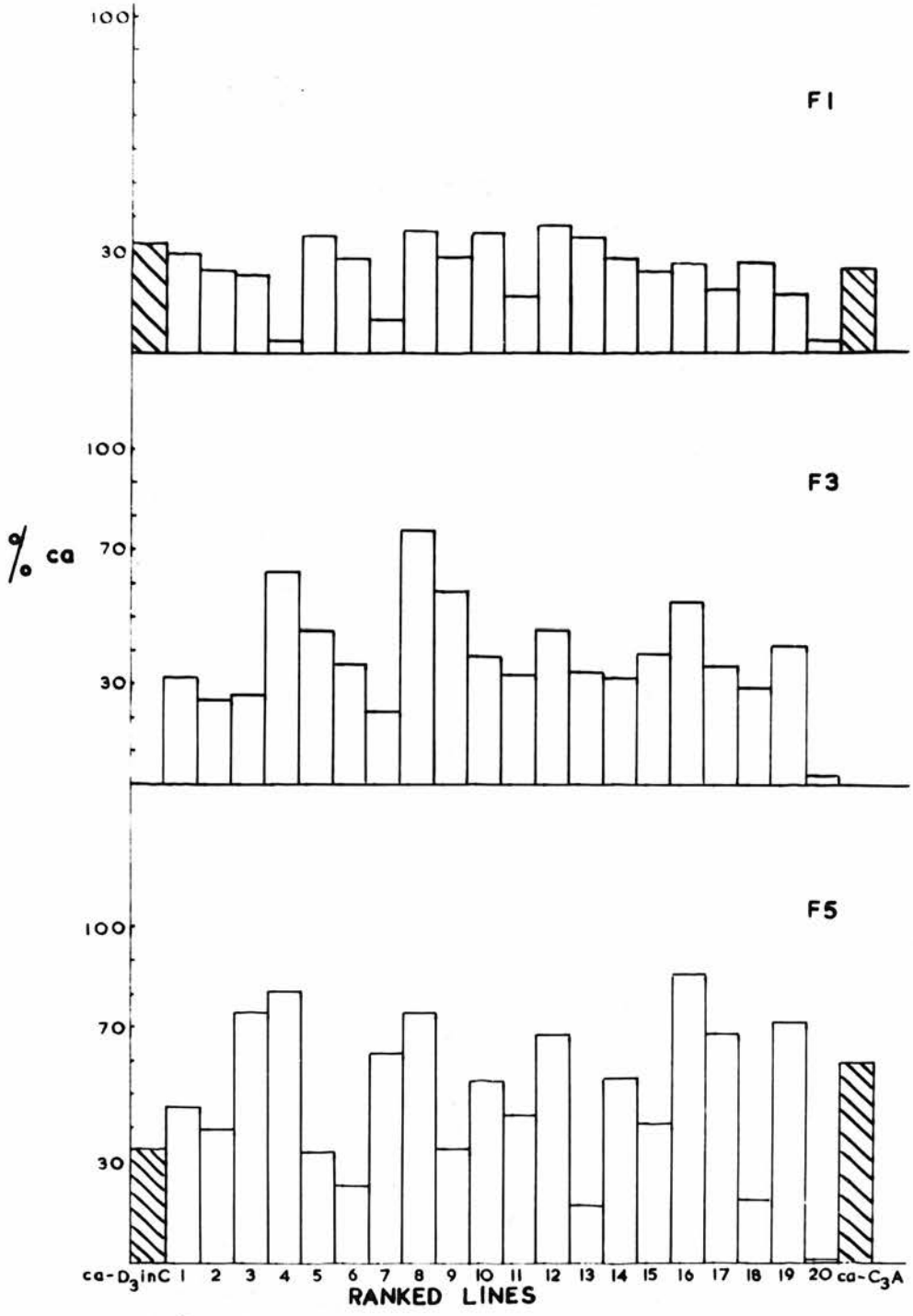


FIGURE 22 COMPETITION BETWEEN ca-BLUE LINES AND Ubx OVER 5 GENERATIONS.

treated with some caution. It may have been more prudent to use a balancer stock in which the inversion prevented crossing-over almost completely.

Conclusions

The relationship between chaeta number and larval competition, attributed to stabilizing selection was observed in an F_2 generation in figure 13. At the observational level, the extremes for chaeta number have been eliminated at the expense of the intermediates. It was suggested in experiment 7 that this relationship could be explained in terms of an environmental effect or of a selective effect or perhaps, more likely, a combination of both. If selection was responsible for restricting the range, this in turn could come about in several different ways. On the basis of the optimum model individuals could have been selected against solely on their chaeta number phenotype. Secondly selection could operate on the degree of homozygosity on the chaeta phenotypes, as the extremes would be more homozygous than the intermediates. The third explanation was that sub-vital genes could have been present in the parent lines in homozygous conditions and are closely linked to chaeta number genes.

The results from experiment 7 suggested that the last explanation is most probable. From the fact that the parent lines differ in hatchability and larval survival it can be concluded that sub-vital genes have reduced the fitness in the high line C_3A to a greater extent than the low line (DF).

The behaviour of the synthetic populations in experiment 8 do not provide conclusive evidence for any one of the explanations of selection.

The change in the Orange and Blue populations could be explained on the optimum model and the Red and Purple would conform to the homeostatic model. However the Blue and Red could also be explained on the basis of sub-vital genes present in the C_3A third chromosome. It is obvious that no one explanation will fit the results and that some unexplained interaction between these chromosomes from C_3A and DF is present.

However it can be concluded from experiments 8 and 9 that larval competition did have an effect on the ca-Blue populations. From experiments 10a and 10b it is found that there is little difference between homozygous individuals differing in chaeta number except for possibly the extreme high individuals. This type of result would fit with the explanation of sub-vital genes being present in the high parent. The reduction in fitness due to sub-vital is likely to be small as the reduction in frequency of the high chromosomes in ca-Blue populations took a considerable time. High chromosomes were still present in the pot cage of ca-Blue even after about thirty generations. It may be that these sub-vitals are maintained in the population through an overdominant effect on fitness. Kearsey & Barnes (1970) reported that females with high chaeta number from a cross between a high and a low selection line produced fewer and less viable offspring in comparison to the rest of the population. They did not test for differences in larval survival within phenotypes over the range. There is further evidence for the presence of sub-vital genes in the high lines from experiment 11. Two of the high scoring lines with about the same chaeta score (47) have different survival rates when competing against Ubx which would suggest that differences other than the number of chaeta are more important.

The main conclusion from the results described in this chapter is that genes controlling the character sternopleural chaeta number do not appear to contribute to the fitness of the individual. Thus genotypic differences in the character do not necessarily cause differences in fitness. However it was found that apparent differences in fitness between chaeta genotypes did occur but the evidence suggests that these differences could be explained in terms of sub-vital genes.

CHAPTER FIVE

PERTURBATION EXPERIMENTS USING WILD POPULATIONS

Experiment 12 Perturbation of gene frequencies in three wild populations

Introduction

One of the main criticisms of the two preceding chapters could be that the genetic material which was used for all the experiments has been derived from only two highly selected sternopleural chaeta lines. These two lines C_3A and DF are likely to be unique and therefore the results and conclusions from experiments could be misleading because the behaviour of these two lines may not correspond to the behaviour of genotypes found in wild populations.

The phenotypic variance of sternopleural chaeta number within wild populations is small and it is difficult to demonstrate differences in fitness over the phenotypic range. Linney et al (1971) using a wild population from Texas, did find differences in fitness over the phenotypic range from the progeny of a large group of males sampled from this population maintained in a cage in comparison to males reared under low density conditions.

Thoday & Gibson (1972) described a simple test for detecting stabilizing selection operating in wild populations. This test was based on Mather's concept of polygenic balance (1943) and they extended this idea from intrachromosomal to interchromosomal balance. These workers have shown that stabilizing selection can be detected in wild populations.

Another approach to this problem and one which has been used here is that of perturbing the frequencies of chaeta number genes.

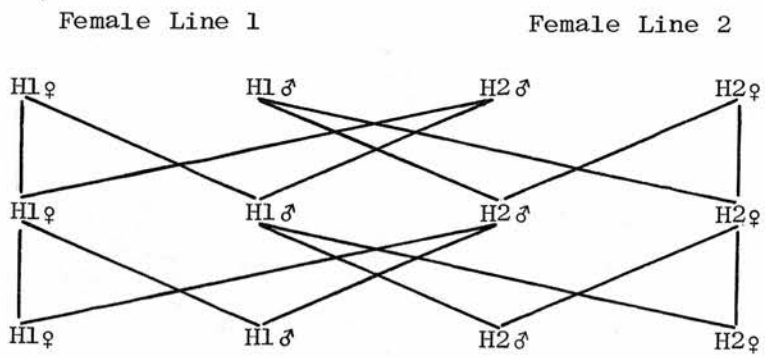
Selection is carried out in order to change the population mean from its equilibrium value but without causing fixation of genes of major effect controlling chaeta number. The population is then relaxed with regard to selection and any return to the original mean under random mating is observed. This type of experiment has been carried out by Latter & Robertson (1962) using the Kaduna population. Selection was carried out in both directions with a selection intensity of 40% (a proportion of 10 being selected out of 25). Selection was relaxed at various times in both high and low lines under crowded conditions. There was no return by the high selected lines but the low lines returned by up to 50%. Robertson (1967) repeated this type of experiment but allowed the relaxed populations to run in population cages maintained on pots for about two years. No return to the original mean was found for either high or low lines, although sufficient variation was present for a return to the original mean as found by back selection.

Since it has been claimed that selection on chaeta number is mediated through larval viability (Linney et al, 1971), the conditions under which selected lines are relaxed will be of importance. It may be that selected lines maintained under intense larval competition will return to the original population value for chaeta number but under non-competitive larval conditions there may be no return as these conditions may support a wide range of fitnesses. This proposal will be examined in this chapter.

Materials and Methods

Three populations were chosen in which selection for sternopleural chaeta number was carried out. The first population was the standard Kaduna which has been maintained in this laboratory for over 25 years as a large random mating population of approximately 5,000 individuals. The second population had been initiated from a sample of around 1,000 adults recently caught at Manzanares in the region of La Mancha in Spain. This population was maintained as a large randomly mating population for about three months before selection was started. The third population was started from a large sample of unknown size caught in Dahomey in West Africa. This population had been maintained in Groningen University in Holland for approximately one year. A large sample of several thousand adults was obtained from there and maintained as a large population for several months before starting selection.

The intensity of selection used was 25%, selecting 12 out of 48 for both high and low chaeta number. Control lines were also set up with 12 pairs of randomly mated individuals. The system of mating used was that of repeated double first cousin. The reason for using this system was that inbreeding is kept to a minimum in the initial generations (Crow & Kimura, 1970). The mating scheme is as follows:-



In each generation reciprocal crosses were made between the two lines in both directions of selection and in the controls. There was no replication of selection lines but during the relaxation period the crossing was stopped resulting in two independent lines.

At the start of the experiment, a sample from each population was reared at low density in a bottle culture. One hundred individuals of each sex were scored for chaeta number. The 24 highest females and 24 highest males were selected and two lines, each of 12 pairs, were set up at random. The same procedure was used for the 24 lowest females and males. Two control lines were set up with 12 pairs each taken from another sample from the low density bottle. All lines were run in bottles and the parents allowed to lay for three days. Four generations of selection were carried out in the high direction but selection was continued in the low line for twelve generations. At generation four, selection was stopped and the two high lines and the two control lines in each population were run separately in bottles at low density, i.e. the randomly mated flies were tipped over into new bottles and allowed to lay for three days. Highly competitive conditions were initiated from the discarded parents of low density bottles and allowed to lay eggs for up to 9 days. The progeny from these high density bottles were tipped over into new bottles after about two and a half weeks and allowed to lay for 9 days before being discarded. In the low line a random sample of 48 flies was removed for the continuation of selection and the rest of the flies were tipped into bottles and relaxed under low and high density conditions.

One hundred flies, 50 of each sex, were scored from each line for each density and for each population in the third generation of

relaxation. The same number was scored at generation 12 but this time the samples from the high density conditions were scored under low density by allowing the flies to lay in extra bottles for a couple of days before setting up the next generation. The same procedure was used when scoring flies at generation 23. In this generation half the number of flies were scored. At about generation 21 small samples were taken from some of the lines and back selection was carried out.

Selection in the low lines was terminated at generation 12. At generation 10, the discarded parents were taken from both lines and first instar larvae were collected from them. Low and high larval densities were set up for three generations using 0.5ml of standard food medium in $1\frac{1}{2}'' \times \frac{1}{2}''$ glass vials. The density conditions were as follows:-

	No. of larvae	No. of vials
Low	10	10
High	50	8

After the third generation, the progeny from both densities were reared under low density conditions and 50 flies of each sex were scored for chaeta number.

The low lines which had been relaxed at generation 12 of selection had been maintained in bottles at low density. These were scored for chaeta number after 11 generations of relaxation. Samples were taken in the next generation for back selection.

Results

The means and variances of the three populations before starting selection are as follows:-

	Mean	Variance
Kaduna	17.40±0.11	2.26
Dahomey	16.55±0.16	4.99
Mancha	18.77±0.16	5.16

Both the recently isolated populations are much more variable than the established Kaduna population. The highest scoring flies in Kaduna have 21 chaetae whereas in Mancha, flies from the same sample size, score 26 chaetae. The means are different between the populations and this appears to be related to body size. Although body size was not scored for either the Dahomey or Mancha population it can be seen that differences do exist. The Mancha flies are the largest, the Kaduna intermediate and the Dahomey are the smallest. These differences in body sizes are considered to be the consequence of differences in developmental time. The egg to adult time was 9 days in Dahomey, 10 days in Kaduna and 11 days in Mancha. The body sizes correspond with the chaeta scores for each population. It is possible that there was some overcrowding in the sample bottle of Kaduna as the mean for generation 0 is lower than both control and low line scores in generation 1.

The response to selection in the three populations is illustrated in figure 23. The total response in the three populations over the four generations of selection for high and low lines and the response after 12 generations of low selection are as follows:-

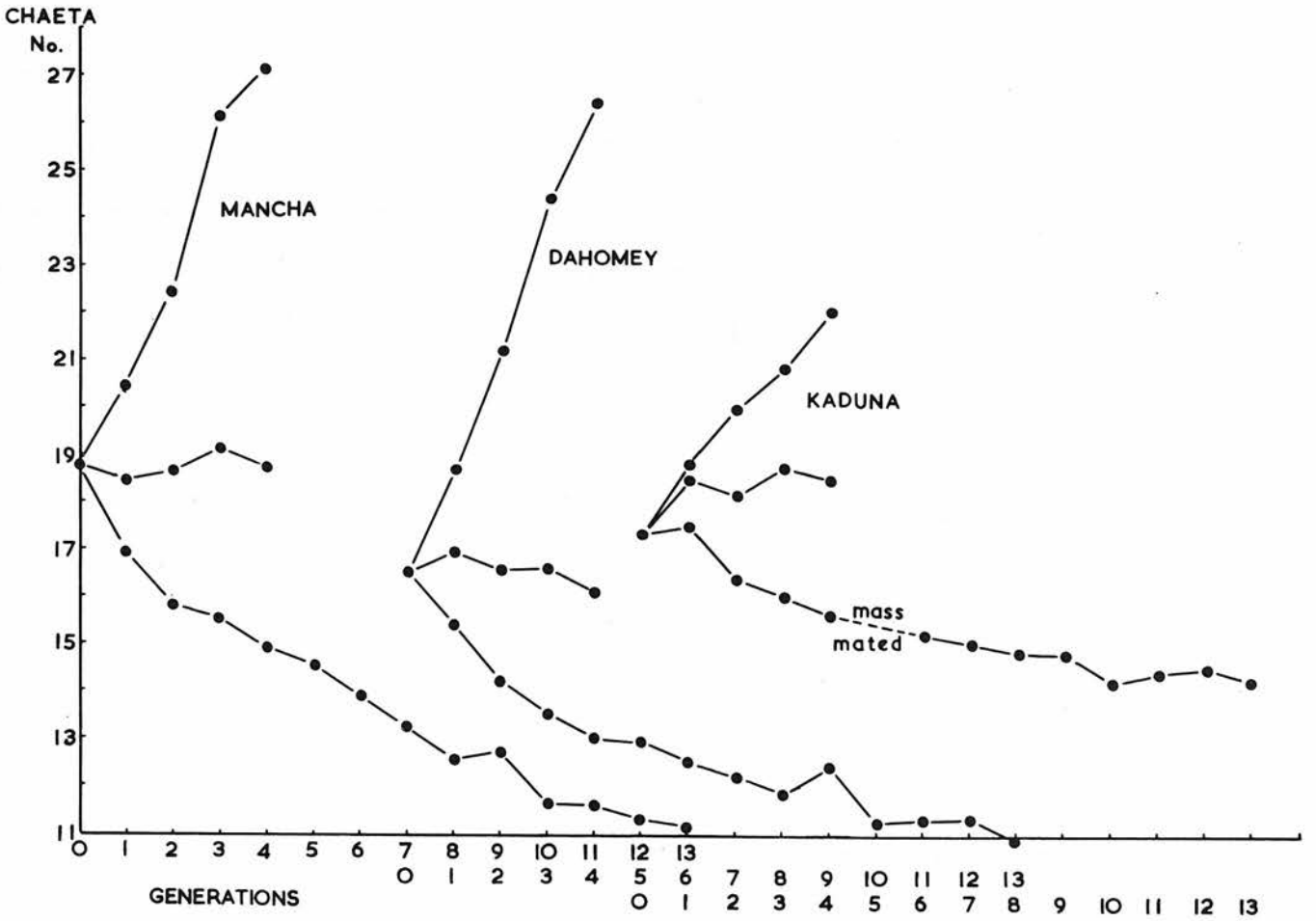


FIGURE 23. RESPONSE TO SELECTION FOR STERNOPLEURAL CHAETA NUMBER.

Generations of Selection

	4		12
	High	Low	Low
Kaduna	4.6988	1.7648	3.0850
Dahomey	9.9133	3.5085	5.6250
Mancha	8.3758	3.8585	7.5900

It can be seen that after four generations of selection, the Dahomey population responded by 9.9 chaetae in an upward direction whereas Kaduna responded by only 4.7 chaetae.

Estimates of realised heritabilities are calculated over the four generations from the regression of cumulated selection differential against response and are as follows:-

	High	Low
Kaduna	0.4759 ± 0.0289	0.3581 ± 0.0661
Dahomey	0.6650 ± 0.0355	0.4497 ± 0.0536
Mancha	0.6379 ± 0.0960	0.3662 ± 0.0568

The Dahomey population has the highest proportion of additive genes controlling chaeta number. Since both Dahomey and Kaduna have similar origins it is possible that Kaduna has decreased in its proportion of additive genetic variation through establishment in a uniform laboratory environment for 25 years.

All three lines continued to respond to selection for low chaeta number in a similar fashion. At generation eight, the three selection lines were kept at $18^{o}C$ for two weeks. This explains the slight increase in chaeta number at generation 9.

a) Relaxation of selection at generation 4

The results of relaxation of the selection lines at generation 4 are illustrated in figures 24-26 for each population. In all the figures both female lines are shown for the generations of selection. There was no crossing between female lines thereafter, each line was maintained separately over the remaining period of relaxation.

It was found previously that when large numbers of adults (c. 50 pairs) were allowed to lay eggs over an eight day period in bottles, larval competition was very high. The distribution of adult body size from a bottle in which an unselected Kaduna population was allowed to lay eggs continuously for 8-9 days was shown in figure 4. The level of larval competition in crowded bottles approaches vial cage conditions fairly well. If chaeta number is associated with larval survival it would be expected that on relaxation, the means of the selected population would return at a faster rate when maintained under intense larval competition than under non-competitive conditions. In figure 24 the changes in the means of the Kaduna selected lines are illustrated. It can be seen that the high lines have returned to the unselected population by a considerable amount in three generations under both levels of larval competition. On the other hand the low lines have changed little in the first three generations of relaxation. Some return would be expected in the first generation of random mating as there will be a high proportion of homozygous individuals among the selected population. Another explanation for the return in the selected lines from the Mancha and the Dahomey populations is the possible effect of inversions present in these populations. From investigations carried out in this

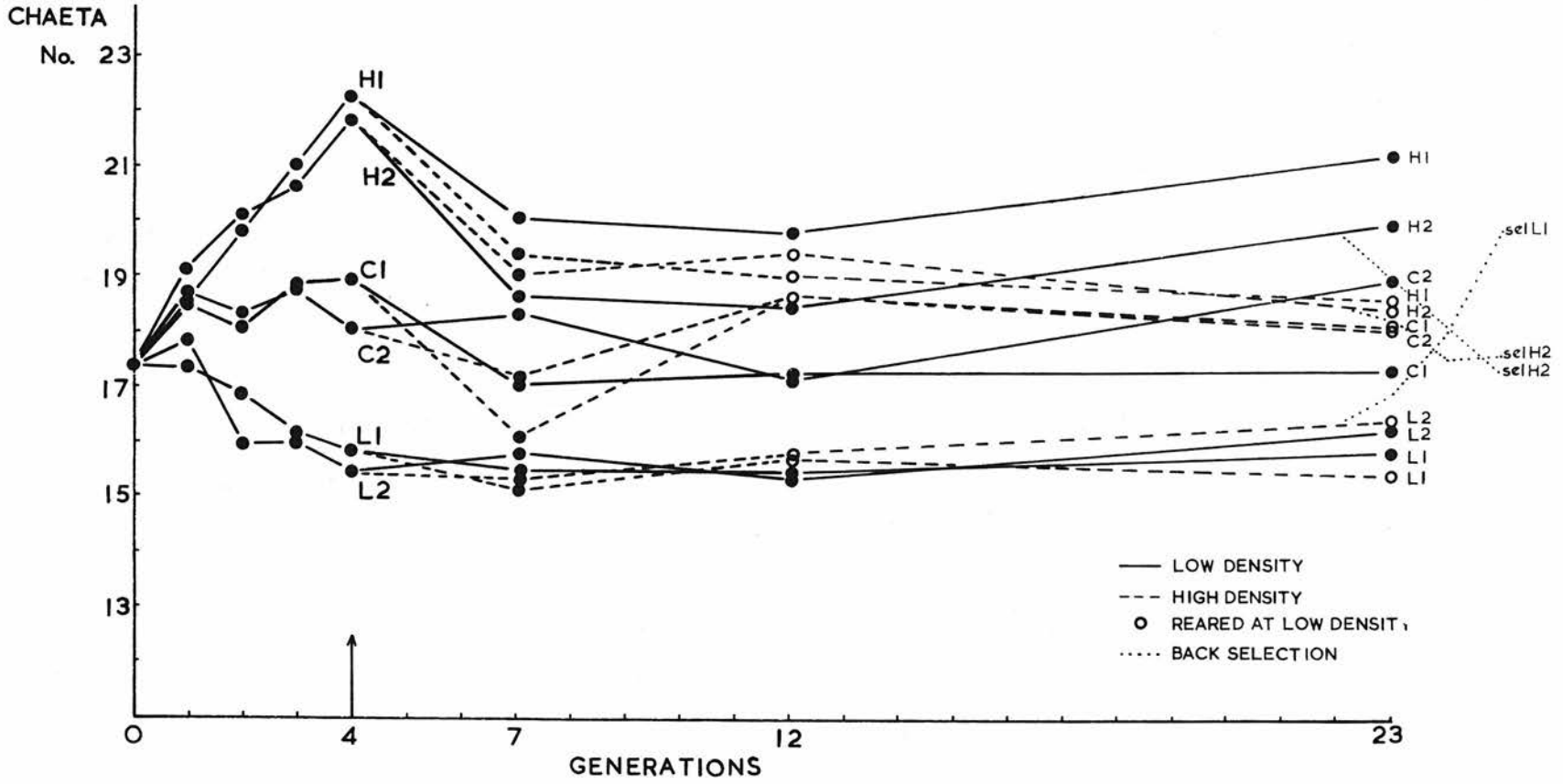


FIGURE 24. KADUNA POPULATION – RELAXATION OF SELECTION LINES AT GENERATION 4.

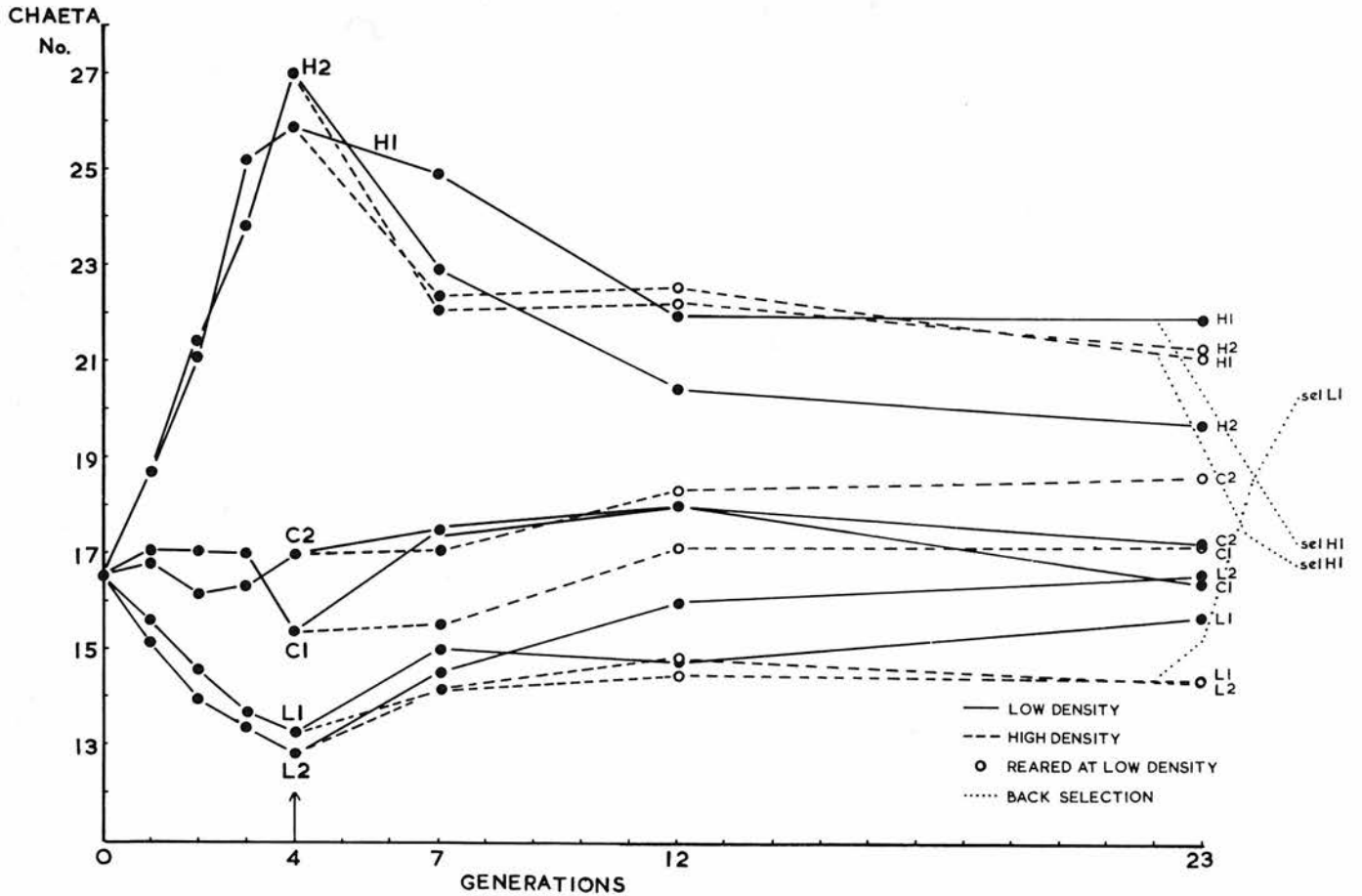


FIGURE 25. DAHOMEY POPULATION – RELAXATION OF SELECTION LINES AT GENERATION 4.

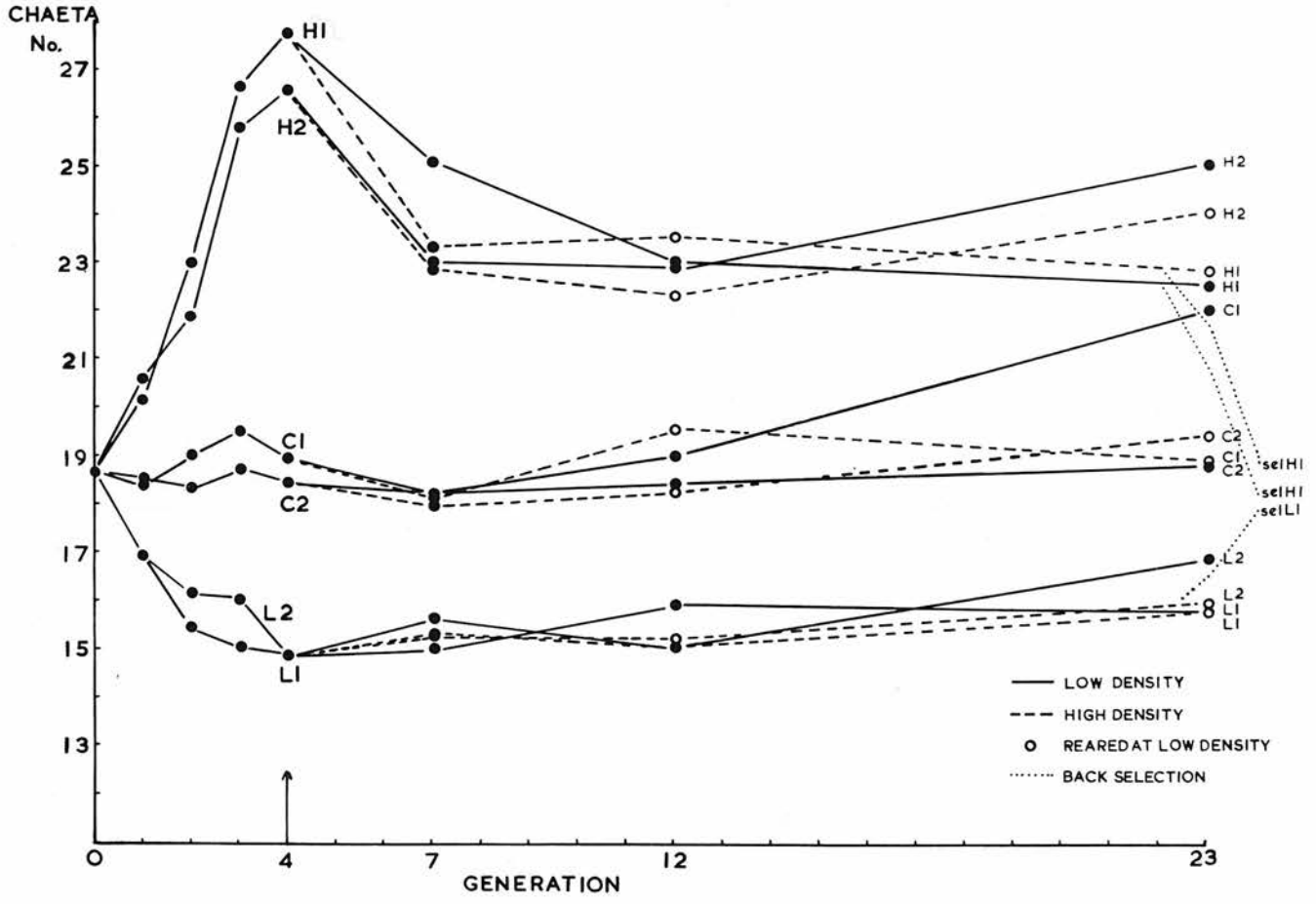


FIGURE 26. MANCHA POPULATION — RELAXATION OF SELECTION LINES AT GENERATION 4.

department it is known (D.A. Briscoe - personal communication) that the Mancha population contains a large number of chromosomal inversion types. It could be that selection for chaeta number has changed the frequency of these inversion types either by chance or by an association with chaeta genes close to or within the inversions. When the lines are relaxed the inversions may return some way back to their previous frequencies and this in turn may affect the frequency of chaeta genes. In the third generation of relaxation, chaeta number was scored on adults sampled directly from the crowded bottles. In this generation the H2 line has a higher score from crowded conditions than from uncrowded conditions. There may be problems inherent in this method of creating highly competitive larval conditions as the numbers of adults per generation may fluctuate. The numbers surviving from high larval competition will not only be reduced but they will have a lower average body size. In the next generation there will be fewer females to lay eggs and the individuals which do lay eggs will be smaller and as a consequence may lay fewer eggs. Therefore this must be taken into account when examining the long term pattern of change in the relaxed lines. The high lines in the Kaduna population have returned to the unselected mean after 19 generations of relaxation under competitive conditions. The difference in the average mean value between the two high lines and the two control lines is only 0.4 chaetae. The high lines maintained at low density have increased in chaeta number by generation 23. It is likely that these changes are due to drift as the sample sizes at low density will be smaller as a consequence of limiting the period of egg laying. The L1 line shows little change over the

entire period whereas the L2 maintained under conditions of high density returned by about 50%. The back selection indicates that sufficient variation still exists in the selected lines for further return to the unselected mean.

In figure 25, the pattern of change under relaxation in Dahomey is illustrated. As with Kaduna there has been some return in the initial generations of relaxation. This return continued in most of the lines up to generation 12 and then the return slowed down. The return has not been faster under competitive conditions. The two low lines at high density have shown little change after the third generation of relaxation whereas the uncrowded low lines continued to show a return. Under non-competitive conditions the low lines have returned to the level of the control lines. It was observed that at all generations, Dahomey maintained very large numbers under competitive conditions. Sufficient variation exists within the selection lines for a return to the unselected mean as indicated by back selection.

The means of the Mancha population in figure 26 show little return to the non-selected mean apart from the initial return. Again larval competition made no difference to the rate of return. The only line which continued to return was L2, maintained under non-competitive conditions. In contrast to Dahomey this population was extremely difficult to maintain under competitive conditions as can be seen from the scores at generation 8. Also small numbers were frequent at low density conditions because of the low egg laying rate in this population. This would account for the increase in score in some of the lines between generation 5, 12 and 23. Back selection has shown

that sufficient genetic variation exists within the lines to allow a return to the unselected mean.

b) Relaxation of low lines at generation 10 of selection

During this experiment it was felt that the competitive effects of larval crowding were imprecise, especially with the Kaduna and Mancha populations. At generation 10 of selection in the low lines, samples of first instar larvae were taken and set up at low and high densities. From the survivors of these controlled conditions further low and high densities were set up. This was carried out for three generations. The scores of the low lines before and after the three generations are given in table 19(a). All the means were scored at low density in bottles. It can be seen that in the Kaduna lines, there has been a decrease in the means at low larval density. At high density the L1 line has returned by 16% but L2 has decreased in score. In both the Dahomey and Mancha lines, there has been a return at both larval densities. The percentage return in the Dahomey L1 line is greater at low larval density than at high and the opposite is found for L2. The Mancha lines show consistently higher returns at high density than at low.

c) Relaxation of low lines at generation 13 of selection

The selection of the low lines was relaxed at generation 13 and the line means are shown in table 19(b). Bottle cultures were maintained over this period under low density conditions although some crowding did occur in some generations. It can be seen from this table that after eleven generations of relaxation, the Dahomey population has returned by 30% in L1 and 23% in L2, Kaduna has shown little change and Mancha has returned by 13% in L1 but L2 has remained unchanged.

Table 19 Chaeta number of low lines after relaxation at generations 10 and 13 of selection

a) Relaxation in vials at generation 10 of selection: controlled larval competition. (Means based on 50♀♀, 50♂♂)

		Gen. 10	Gen. 14	
			Low Density	High Density
Kaduna	L1	14.33*	14.28 (-)	14.81 (16%)
	L2	14.19	14.11 (-)	13.79 (-)
Dahomey	L1	11.31	11.95 (12%)	11.83 (10%)
	L2	11.22	11.52 (6%)	11.86 (12%)
Mancha	L1	11.44	11.82 (5%)	12.31 (12%)
	L2	12.09	12.32 (3%)	12.83 (11%)

* Standard errors = approx. 0.10

( ) % return to unselected level

b) Relaxation in bottles at generation 13 of selection and back selection. (Means based on 25♀♀, 25♂♂)

		Gen. 13	Gen. 24	Back selection	Gen. 29 (unselected)
Kaduna	L1	14.34*	14.22 (-)		
	L2	14.28	14.44 (5%)	15.14 (2 gens)	15.06 (20%)
Dahomey	L1	11.04	12.68 (30%)		
	L2	10.80	12.14 (23%)	14.46 (4 gens)	11.94 (-)
Mancha	L1	11.10	12.08 (13%)		
	L2	11.26	11.36 (1%)	12.13 (3 gens)	11.74 (5%)

* Standard errors = approx. 0.15

In each population, back selection was practised in each L2 line. The Kaduna line responded in two generations by 0.7 chaetae, Dahomey responded by 2.32 chaetae in four generations and Mancha by 0.77 chaetae in 3 generations. The back selection had to be terminated through lack of time but it is assumed that sufficient genetic variation is present for a return to the unselected level. This is supported by the fact that the Kaduna L2 line has returned a further 20% by generation 29 under relaxation although the Dahomey line has decreased by 3%.

Discussion

The most interesting result to emerge from this experiment is the fact that the selection lines from the three populations have behaved under natural selection in a different manner. The Kaduna selection lines have returned by a considerable amount under larval competition in comparison to lines maintained under non-competitive conditions. The fact that the high lines returned to the unselected mean is unexpected. Latter & Robertson (1962) found that when selection was carried out for chaeta number in both directions the competitive index, a measure of fitness in terms of viability and mating ability, was reduced to a greater extent in low lines (50%) than in high (35%). They reported returns in the low lines of 50% and only 14% in the high under crowded conditions. The reverse situation appears to have occurred here, only the high lines have returned to the unselected mean. This would argue very strongly that genes controlling high chaeta number are associated with fitness and that this relationship is dependent on larval competition.

When the two more recently isolated populations are examined, the conclusions are not so clear. In the Dahomey lines there has been some return in the means but it is the low lines which have returned the most. However there is no connection between competitive conditions and the rate of return. In fact the low lines maintained under non-competitive conditions have returned by the largest amount. Neither the high nor the low lines in the Mancha population have returned to any substantial degree except in the case of the high larval densities relaxed at generation 10.

In this type of experiment it is impossible to distinguish between cause and effect. Random changes may affect a very large number of loci and therefore overall fitness may be reduced early on in such experiments as found by Latter & Robertson (1962). However it is possible to compare the behaviour of the chaeta number character in the three separate populations. There is no consistent pattern among the populations. The overall conclusion is that larval competition has had no effect on the return to the unselected level except in the high lines of Kaduna. In all other cases the best indication of a change due to natural selection is given by the line means under competitive conditions as the numbers sampled will be very much larger than the non-competitive conditions and so the effects of genetic drift will be less. The pattern in the Dahomey and Mancha populations are now more similar. The means and variances of the lines for each population under competitive conditions at generation 23 from figures 24-26 are as follows:-

		Base	High		Control		Low	
			H1	H2	C1	C2	L1	L2
Kaduna	$\bar{X}$	17.40	18.62	18.46	18.18	18.10	15.46	16.44
	V_x	2.26	2.04	4.50	3.46	1.64	1.80	2.41
Dahomey	$\bar{X}$	16.55	21.16	21.38	17.26	18.70	14.44	14.40
	V_x	4.99	16.79	14.24	7.91	6.95	1.72	2.49
Mancha	$\bar{X}$	18.77	22.88	24.08	18.94	19.46	15.84	15.96
	V_x	5.16	8.92	11.18	3.40	4.58	1.93	1.79

After 19 generations of relaxation, the selection lines have continued to maintain chaeta number means at about 1.5 standard deviations from the mean of the controls although there is sufficient variation available for a return to the unselected level. The only exception to this is the Kaduna high lines which have both returned to the unselected level. The variation within the lines differs between and within the populations. The high lines from the Dahomey population still contain a large amount of variation. After this period of relaxation, phenotype classes range from 13 to 31, which would indicate that there can be little difference in fitness associated with chaeta number in the Dahomey population. Similarly in the high lines from the Mancha population there is a wide range of phenotypes which have been in competition over 19 generations without any apparent differences in fitness. The Kaduna high lines have behaved in a different manner in that the high scoring phenotypes have been eliminated at the expense of the individuals nearer the mean. The higher variance of the H2 line is attributed to a larger proportion of low scoring individuals in comparison to H1. The different pattern of behaviour between the wild Dahomey and Mancha populations and the

laboratory Kaduna population could be explained in terms of their differences in variation shown for the character. It is possible that the loss of variation from a population will decrease its ability to cope with internal changes brought about by artificial selection. It is known that the longer the length of time a population has been maintained in the laboratory the more uniform it will become (Anderson et al, 1972). The Kaduna population originally contained several chromosome inversions but these have been lost during its laboratory establishment, (Latter & Robertson, 1962). It has also been found in this department that there is a high correlation between the age of a laboratory population and the incidence of segregation at enzyme loci, (Briscoe & Malpica - unpublished). It could be argued that the reason why the Kaduna high lines have returned to the unselected mean is because this population has lost a certain proportion of its variability and is therefore less well buffered to small changes in its internal environment.

It can be concluded from the behaviour of selection lines in the two recently isolated wild populations that chaeta number is not closely associated with fitness.

CHAPTER 6

FINAL DISCUSSION AND CONCLUSIONS

In this final chapter the main conclusions from the experimental results are discussed in relation to other published findings.

Although the optimum model of stabilizing selection has been presented as a mechanism for maintaining variation in the character sternopleural chaeta number (Mather, 1953; Gale & Kearsey, 1968), until recently little in the way of experimental evidence had been provided. In 1970, Barnes & Kearsey published two papers in which they examined a wild population of D. melanogaster recently caught at Austin in Texas. Previous investigations had been confined to artificial populations constructed from inbred lines (Mather, 1961; McGill & Mather, 1972; Barnes, 1968 and Killick, 1970). Kearsey & Barnes (1970) pointed out that populations produced from inbred lines of differing origins might produce misleading results as they would not be relevant to natural populations.

In their first paper Barnes & Kearsey (1970) described the genetic architecture of sternopleural chaeta number in the Texas population. They found, as Robertson (1964) had before, that extensive genetic variation exists for this character. Considerable dominance in the direction of low chaeta genes was found on the arithmetic scale. In the present investigation it was observed in experiment 7 that the average F_1 value between the Kaduna selected lines, C_3A and DF, deviated by 11 chaetae from the midparent value in the direction of the low parent (DF). However, if a log scale is used then the dominance is almost completely removed. On a model of stabilizing selection it would be expected that gene control would be mainly additive with weak ambidirectional dominance. On the other

hand Wolstenholme & Thoday (1963) found two completely dominant genes for high chaeta number on the third chromosome in their population.

Barnes & Kearsey (1970) deduced from information on the Texas population that around 16 loci were responsible for controlling variation in chaeta number. However, it is not the total number of loci which is of importance, but the location of these loci on the chromosomes and the magnitude of their individual effects. From extensive work carried out by Robertson (1970) and by Spickett & Thoday (1966) it is concluded that less than 10 loci control around 80% of the variation. In addition it has been found that about 60% of these effects are located on the third chromosome. It is likely that a larger number of loci contribute to this character but in practice an estimate of the upper limit may be very difficult to obtain. The assumptions which Barnes & Kearsey make of equal effects and equal gene frequencies are considered to be unreasonable in the context of the work carried out by the above authors.

In the second paper (Kearsey & Barnes, 1970) a synthetic population was constructed from a high and a low selection line, both originating from the Texas population. This population was maintained on a rotational vial system and after 6 months female chaeta scores from the cage and a low density sample were as follows:-

	Low	Cage
mean	21.33	18.47$\pm$0.10
variance	35.17	8.23

Males were progeny tested from both cage and low density conditions and the regression estimates of offspring on male parent were:-

Cage ♂♂	0.4168±0.0666
low density ♂♂	0.3833±0.0503

It was concluded that there had been a selective elimination of extreme genotypes, since there was no significant difference between the regression estimates. Since the restriction of the phenotypic range was attributed solely to a selective effect, the depression of chaeta number due to larval competition was assumed to be constant over the phenotypic range. However, it was found in this present investigation that the environmental depression of chaeta number was not constant but changed by a factor of 3 over this same range. By correcting their distribution with this differential factor a different distribution of relative frequencies is produced. Fewer of the extreme high phenotypes have been eliminated. A more satisfactory explanation would be that both environmental and selective effects have contributed in a similar manner to the restriction of the phenotypic range. Although the regression lines are not significantly different, the estimate from the cage males is numerically larger than low density indicating a possible environmental effect. It is doubtful whether this experimental design would in fact be sensitive enough to distinguish between the environmental and selective effects. Some of the drawbacks inherent in this design were discussed in experiment 10. Since the optimum value does not lie in the centre of the range, due to the scale of measurement, the regression slope from progeny tested cage males will be affected to a greater extent by the high scores than by the low scores. Also it can be seen from the estimates of the error variances that the cage males have a much larger error as a consequence of the variation in body size. This increased error variance from cage

males will also decrease the sensitivity of the test.

Since the population used by Kearsey & Barnes (1970) was an artificial population constructed from two selection lines originating from eight flies from the Texas population, it is probable that there would still be a considerable amount of linkage disequilibrium. These authors mention that in the selection programme the original lines were difficult to maintain thus suggesting that sub-vital genes may have been present. Thus in the population constructed from these lines extreme individuals would be expected to carry a higher proportion of sub-vitals in a homozygous condition than intermediate scoring individuals. Thus a priori it would be expected that the extreme individuals would not survive competition because of the contribution of sub-vital genes. It was found that the lines C_3A and DF used in this investigation had a lower larval survival than crossbred progeny. Also the high selection line, C_3A did worst of all in comparison to DF and other lines. This would fit with the data of Kearsey & Barnes (1970) in that a higher proportion of extreme high scoring individuals were eliminated in their artificial cage population than low scoring individuals. However, Kearsey & Barnes state that a distribution based on heterozygote advantage would not fit the distribution of relative fitnesses. As their distribution of relative frequencies depends partly on the correction factor used for cage individuals and since their factor is wrong, it is likely that their final distribution of relative fitnesses is misleading. Therefore this evidence does not substantiate their final conclusion that the optimum model of stabilizing selection is a valid explanation of their data.

Kearsey & Barnes (1970) list the means and variances of seven wild populations from widely different geographical origins and comment on their similarity. However, Thoday (1958), whom they quote, stated that "different populations of a species have different characteristic chaeta numbers" (the emphasis is this author's) suggesting that chaeta number shows adaptive significance. Under different environmental conditions it might be expected that chaeta number would be adapted to different situations. This was demonstrated by Beardmore (1956, see Thoday, 1958) and Parsons (1961). Beardmore found that flies reared at different temperatures (20°C, 25°C and 30°C) showed changes in their chaeta numbers over a period of 30 generations. At 20°C chaeta number increased, at 25°C it stayed constant and at 30°C it decreased. Parsons obtained the same results using 25°C and 30°C environments over a single generation. He also found that body weight and developmental time decreased at 30°C as did chaeta number. Thus the change in chaeta number may be explained as a consequence of a change in another related character, namely body size, which in turn will depend on developmental time. In the three wild populations used in chapter 5 in this investigation chaeta number is related to body size. In the two recently caught populations used in this investigation the population from Dahomey (latitude 10°) has a chaeta number of 16.56. In comparison the population from La Mancha (latitude 40°) has a chaeta number of 18.77. Since the Kaduna population has been maintained at 25°C for around 25 years its chaeta number may have increased with a change in body size, as directional selection for shortening development time would have been relaxed. These findings would suggest that differences in body sizes are a consequence of developmental time, which is

likely to be closely associated with fitness. Chaeta number can be regarded as a consequence of changes in other characters and is itself neutral in the adaptive sense. Kearsey & Barnes's statement on the "remarkable similarity" of the seven populations is then in contradiction to what Thoday (1958) has stated.

A second point mentioned by Thoday (1958), and again quoted by Kearsey & Barnes, is that extensive genetic variation exists for chaeta number in all populations so far studied and yet the observed phenotypic variation is very small. Thus it is concluded that stabilizing selection must be operating to continually eliminate extreme deviants. It is implied that stabilizing selection will maintain variation for this character. On the model of heterozygous selection this might be true, although variation would not be maintained indefinitely. Alternatively the optimum model is unlikely to maintain variation as fixation of genes conferring the optimum value is the most likely outcome (Robertson, 1956). The experimental evidence would suggest that when selection for intermediate values of a quantitative character is carried out, there is a decrease in genetic variance (Prout, 1962; Rendel, 1960; Scharloo, 1964 and Thoday, 1959). This would imply that chaeta number is not under selection.

In a third paper (Linney, Barnes & Kearsey, 1971) evidence of stabilizing selection was presented from experimental evidence using the original Texas population. Large samples (250) of males were collected from a cage population and from a low density sample and both were scored for chaeta number. After two days all surviving males were mated to a tester stock. It would appear that of the males sampled from the cage about half died before mating as did about a

quarter of the males from the low density sample. It is not clear whether the chaeta scores given are from the original samples or from the survivors of the samples. Also it is not mentioned whether the cage males were virgins or a sample of flying males. If it is the latter then it is possible that differences in the genotypic arrays from the cage and low density samples may be present at the start of the experiment. O'Donald (1971) found that when flies were kept in cages containing different amounts of food, there was an elimination of extreme scores among males in the cage where there was less food, although this was not found for females. The scores of males from the cages from O'Donald's data were:-

Amount of food per cage	Mean	Variance
20 vials	17.71	4.59
6 vials	17.57	3.57

Therefore some other factor apart from larval survival may have caused a difference between the two groups of males.

However, Linney et al (1971) point out that heterotic selection could also explain the results from their experiment and also the previous experiment using the F_2 population (Kearsey & Barnes, 1970). In an attempt to conclusively prove the validity of the optimum model they set up a second experiment using homozygous lines differing in chaeta number. The chaeta scores of the lines were 13.41, 16.75, 17.20 and 21.69. It was found that under high larval competition the extreme phenotypes from the low and high lines did not survive. This is in marked contrast to the results found in this investigation in experiment 10 where no difference in larval survival could be detected

for the majority of the lines used. A much more extensive investigation of the lines used by Linney et al (1971) would seem worthwhile to decide conclusively that larval survival was contributing to these differences. It might be that differences in oviposition rate would affect the outcome of such an experiment. Although they used only four lines i.e. two extremes and two intermediates, the results do indicate that the optimum model could be a valid explanation of stabilizing selection.

From the results of the present investigation little evidence has been found to substantiate the optimum model of selection. In experiment 8 and 9 the means of the ca-Blue population declined from a value of 27.5 to 21.5 which resulted in an increase in frequency of low chromosomes. However, no differences in larval survival could be found among homozygous lines extracted from this population in experiment 10, although there may have been selection against the highest lines representing the original high parent. This could be explained by the presence of sub-vital genes lowering larval survival. In experiment 11 no association between chaeta number and competitive ability were found. These results would indicate that the decrease in mean in the ca-Blue populations was a result of a lower viability of high chromosomes rather than a selective advantage of the low chromosomes as a consequence of an interaction between chaeta number and fitness. The only unexplained result is that from the Orange and Purple populations in experiment 7 where there was an apparent increase in the mean of the Orange population indicating that genes for high chaeta number on the first, second and fourth chromosomes have increased in frequency, whereas no change occurred in the Purple population. The

results from these populations cannot be explained on a simple model of selection.

Barnes & Kearsey (1970) comment that "It is known that the low and in particular the high selection lines regress when artificial selection is relaxed, ...". The low lines in their selection programme returned by 1 chaeta before selection was continued. Similarly it was found that in the high lines a return was observed on relaxation of selection, but was confounded with a temperature effect. Thus the magnitude of the return is not provided. It was implied that chaeta number was returning under the influence of natural selection. An immediate regression to the mean would be expected but not necessarily as a consequence of selection on chaeta number. This return would be related to the proportion of heterozygous loci per individual in the selected populations. The homozygous individuals having high or low values for the direction of selection are likely to be at a disadvantage over the more heterozygous individuals through the effect of sub-vital genes. Barnes & Kearsey (1970) do not report any results for long term relaxation experiments of selected lines. In experiment 12 of this present investigation the Kaduna population would certainly fit with evidence of the optimum model of selection, but this does not hold up for the other two recent populations. It is concluded that the return in the Kaduna population reflects a decrease in stability through loss of variability.

Throughout their investigation it has been assumed by Linney et al (1971) that intense larval competition is the norm in natural situations. There is no evidence, concerning D. melanogaster to suggest that this extreme larval mortality occurs frequently in

nature. Birch & Battaglia (1957) concluded from a survey of breeding sites of D. willistoni that climatic conditions such as high temperatures and drying out of sites killed off large numbers of larvae and that mortality was not due to absolute food shortage. They also found the same results for D. simulans. Sokoloff (1957), who observed the breeding sites of D. pseudoobscura, persimilis and miranda, concluded that density dependent effects were not important for various reasons such as interference by other organisms, predation, and weather conditions. Basden (1972) records around 20 species of Hymenopterous wasps known to parasitize Drosophila species in natural populations.

On the other hand McFarquhar & Robertson (1963) found a large variation in body size in wild D. subobscura in comparison to laboratory scored individuals. It was concluded by these authors that this variation could be attributed mainly to nutritional deficiencies of breeding sites rather than as a consequence of larval competition. Similar evidence was found in D. disticha (Robertson et al, 1968) and it was concluded from field observations that larval competition was not an important source of food shortage. However, these investigations do not provide information on the proportion of larvae which survive to adulthood.

From the present investigation it was found that wild flies brought back from Spain showed little signs of having experienced food shortages. All the surviving adult females were measured for thorax length and in comparison a sample of laboratory reared adults from a pot cage were also measured at a later date. The two distributions are illustrated in figure 27. Some reduction in body size will have

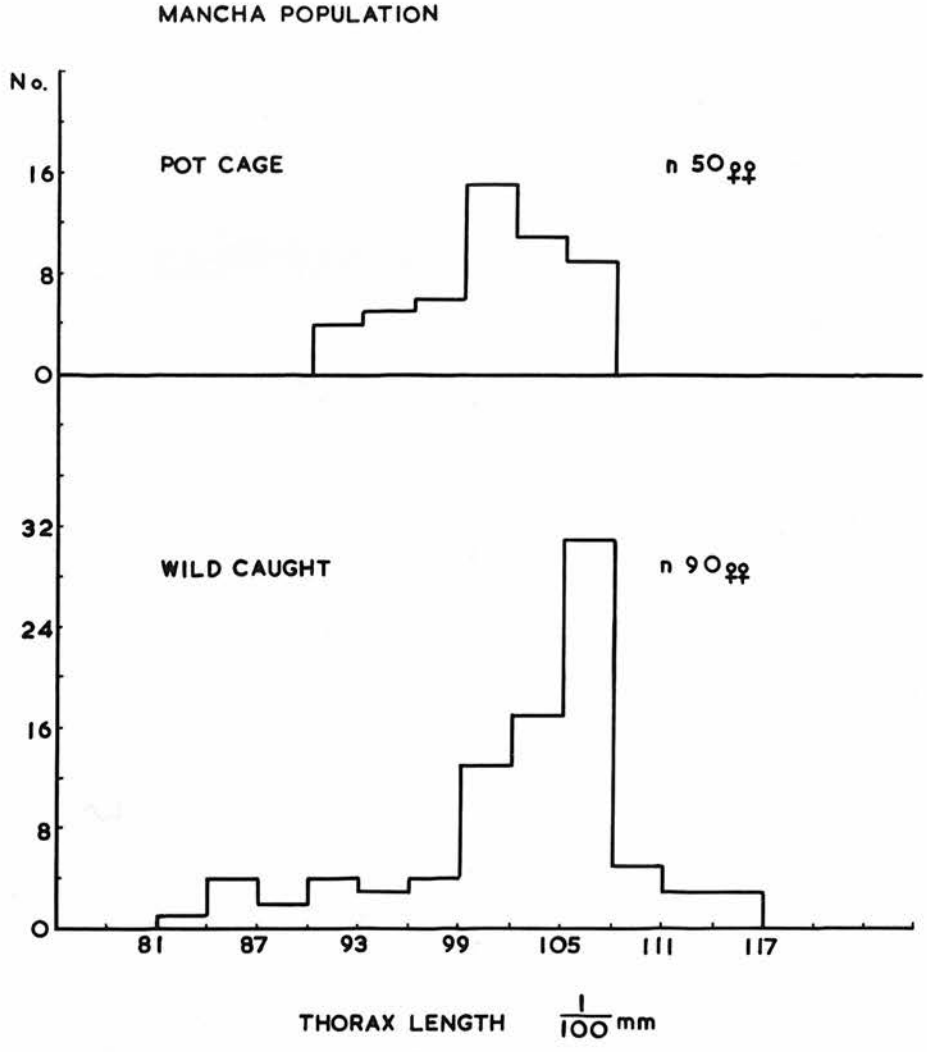


FIGURE 27. A COMPARISON OF BODY SIZE BETWEEN FLIES CAUGHT IN THE WILD AND LABORATORY REARED FLIES.

occurred under pot cage conditions as was found in the Kaduna cage system. It is likely that average body size of flies reared under low density conditions and those of wild flies in this case would have about the same mean. Although the range in body size is greater for the wild flies, it is likely that this could be due to nutritional and temperature variations. However, from this distribution of wild flies it appears that the majority of individuals have experienced little or no effect of food shortage. As these Spanish flies were collected in the Autumn of 1972, it might be expected that the population would be at its highest peak and if larval competition did occur, it would be at this time of year. However, it is an estimate of the percentage mortality which is of importance, but this type of information would be extremely difficult to obtain. As far as is known there are no reports of intense larval competition having been observed. On the contrary Sokoloff's observations indicate the opposite.

If it is presumed that high mortality occurs at the larval stage through competition or for some other reason and it is assumed that the range of chaeta number is restricted through selection, then the observed variance of wild flies should be lower than laboratory reared flies. Wild caught females from Spain together with laboratory reared females under low density conditions were scored for chaeta number:-

	Wild	Laboratory
Number	223	100
Mean	19.02	19.19
Variance	5.58	3.79

These scores indicate that selection has not restricted the phenotypic range under wild conditions. On the contrary it is more probable that environmental influences have increased the variation in chaeta number.

Finally it is necessary to ask two questions. First, has the evidence from this thesis and from previous work by others contributed to the understanding of the role of metrical characters in natural populations? Secondly, is the method of investigation which has been used here the most useful approach for the study of such characters in evolution?

Considering the first question, it has been found from this thesis that genotypes differing in the number of chaeta on the sternopleuron do not exhibit differences in fitness as measured by egg to adult viability. This is in contrast to the observations of Linney et al (1971) who found large differences between inbred lines differing in chaeta number. It must be pointed out that both investigations have been carried out on artificially constructed populations and within artificial environments. However, it is concluded from this present investigation that under these conditions chaeta number is a trivial character of little importance to fitness. It is likely that other metrical characters such as abdominal chaeta number, wing length and body size are similar in their effects on fitness (Falconer, 1964).

However it is necessary to verify the conclusions found in the present investigation with observations under natural conditions since there is still little information on the ecology of the pre-adult stages particularly in D. melanogaster. As has already been pointed

out, if selection is operating on chaeta number then a smaller variation in the character would be expected in wild individuals than in comparison to individuals reared under laboratory conditions. Thus an examination of the spatial and temporal aspects of the environment on the variability of chaeta number would be a necessary next step in the understanding of the relevance of the character. It is possible that variation in this character can be accounted for by recurrent mutation and heterozygote superiority (Latter, 1960). Alternatively this variation could be maintained through some pleiotropic effect on a character important in fitness. From this investigation it was found that larval survival was not associated with chaeta number as was claimed (Linney et al, 1971). By studying the character in its natural environment it may be possible to find some fitness character closely associated with chaeta number. Until this has been investigated it can be assumed that the explanation of mutation and heterozygote advantage can account for the maintenance of variation in the character.

In answer to the second question concerning the rationale of the method of study it is certain that conclusions about the adaptation of the character will be wrong. Selection does not operate on single characters such as chaeta number but through the capacity of the genotypes to leave more offspring relative to co-existing genotypes. Successful genotypes are the products of selection through the formation of an integrated pattern of development which is itself buffered against changes in the external environment. Therefore to understand the contribution of the genes controlling chaeta number we need to know the contribution of these genes throughout the entire life

history of the organism. However as Dobzhansky (1956) has stressed we can only observe the difference in two gene substitutions on the organism and therefore it is difficult to determine the total contribution of any one substitution. In the case of metrical characters not only are there different substitutions at individual loci but there are perhaps ten loci to consider. Also we do not know whether each of the loci which affect chaeta number have similar types of pleiotropic effects or whether they individually contribute to widely different processes.

It is not surprising then that the study of quantitative characters has been oversimplified in any attempt to obtain simple explanations. However, this approach will not increase our understanding of the process of evolution. We must attempt to take a broader and more comprehensive view of the process of evolution of genetic systems.

A more rational approach would be to utilize the information obtained from the extensive studies on location of quantitative genes. Thus by locating single loci and isolating them in otherwise isogenic lines it might be possible to study the differences between the loci in their effect on the developmental processes. By isolating all the detectable loci some pattern may emerge which would indicate the role of chaeta number genes in the developmental pattern. However, it may be that until the basic control of differentiation and development is understood our comprehension of the evolution of the genetic system must be limited.

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"I've nothing against you personally— I just don't like that damn geneticist taking me for granted."

Punch 1965

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